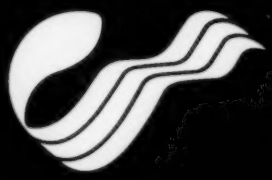

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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The Stockholm Water Prize and the Experimental Lakes Area

Guest Editorial

On April 15, 1991, the Stockholm Water Foundation announced the awarding of the first Stockholm Water Prize to Dr. D. W. Schindler, who led the Experimental Lakes Area (ELA) program for its first two decades. This prize, which has a monetary value of $150 000 US, will be formally presented by Her Majesty Queen Sylvia on August 14, 1991, as part of the Stockholm Water Symposium.

The letter accompanying the announcement stated that "The prize which is awarded for the first time is given to you in recognition of your outstanding research regarding eutrophication and acidification of lakes, research that has led to positive changes in environmental legislation in Canada, the USA and the EEC." Dr. Schindler's contributions to the climate change debate were also acknowledged. A recent paper in *Science* (November 16, 1990) on the effects of a two-degree warming in the ELA has already had a tremendous impact on the international climate-change debate. It clearly demonstrated the value of the integrated long-term monitoring function that has been built into the ELA program. The ELA team has consistently provided global leadership in the development of whole-ecosystem research on the cumulative impact of human activities on freshwater ecosystems. In the process the team has also demonstrated that long-term whole-ecosystem research can be most effective in identifying and justifying policy and legislative imperatives.

We have reason to be proud that the first Stockholm Water Prize was awarded to the person who has led Canada's foremost freshwater research program. This prize is also a major honor for those visionary pathfinders who created the ELA program. The Fisheries Research Board of Canada (FRBC) sponsored and mandated the program in its formative years. The Department of Fisheries and Oceans has, through its continuing support of the program, made a major contribution to the international effort to protect, restore, and manage freshwater ecosystems and freshwater habitats. The awarding of the first Stockholm Water Prize to Dr. Schindler provides a clear indication of the international recognition given to his work at the ELA. For people familiar with the ELA program and Dr. Schindler's remarkable contribution to the program, the announcement was most welcome, but hardly surprising. Many of us have long considered this unique program as being at the vanguard of global efforts to understand and protect freshwater ecosystems. The announcement is, however, most timely, coinciding with a major effort to strengthen the ELA mandate and to ensure a funding base that is adequate for program renewal and revitalization.

The Stockholm Water Prize is the latest example of the international recognition that the ELA program and Dr. Schindler have received. External reviews have invariably given the program top marks, and Dr. Schindler has himself received numerous awards and medals from scientific associations. The Experimental Lakes have also been featured in the popular press and on film, television, and radio. Examples include *Newsweek, U.S. News and World Report, New York Times, Washington Post, Toronto Globe and Mail, Toronto Star, Ottawa Citizen*, and the *Winnipeg Free Press*.

Le Prix d'hydrologie de Stockholm et la région des lacs expérimentaux

Collaboration spéciale

Le 15 avril 1991, la Fondation d'hydrologie de Stockholm annonçait l'attribution du premier Prix d'hydrologie de Stockholm au D[r] D. W. Schindler, qui a piloté le programme de la région des lacs expérimentaux (ELA) pendant ses deux premières décennies. Ce prix, d'une valeur monétaire de 150 000 $ US, sera présenté officiellement par Sa Majesté la Reine Sylvia, le 14 août 1991, dans le cadre du Symposium d'hydrologie de Stockholm.

La lettre accompagnant l'annonce précisait : « Ce prix, qui est attribué pour la première fois, vous est décerné en reconnaissance de vos travaux exceptionnels de recherche sur l'eutrophisation et l'acidification des lacs, qui sont à l'origine de changements positifs à la législation environnementale au Canada, aux États-Unis et dans le CEE. » La lettre reconnaissait également les contributions du D[r] Schindler au débat sur le changement climatique. Un récent article paru dans *Science* (16 novembre 1990) sur les effets d'une augmentation de deux degrés de l'ELA a déjà d'immenses répercussions sur le débat international que suscite le changement climatique. Il démontre clairement la valeur de la fonction intégrée de surveillance à long terme dont est assorti le programme ELA. L'équipe ELA n'a pas cessé d'assurer un leadership global dans le développement de la recherche panécosystémique sur l'effet cumulatif des activités humaines sur les écosystèmes d'eaux douces. L'équipe a aussi établi, pendant ce temps, que la recherche panécosystémique à long terme peut être un moyen très efficace de définition et de justification des politiques et des mesures législatives à adopter.

Nous avons de quoi être fiers que le premier Prix d'hydrologie de Stockholm ait été décerné à la personne qui a piloté le plus grand programme canadien de recherche sur les eaux douces du Canada. Ce prix est aussi un grand honneur pour les éclaireurs visionnaires qui ont créé le programme ELA. L'Office des recherches sur les pêcheries du Canada (ORPC) a parrainé et mandaté le programme dans ses années de formation. Pour sa part, le ministère des Pêches et des Océans a, par son appui constant au programme, contribué vigoureusement à l'effort international de protection, de restauration et de gestion des écosystèmes et des habitats d'eaux douces. L'attribution au D[r] Schindler du premier Prix d'hydrologie de Stockholm est une indication claire de la reconnaissance internationale à laquelle il a droit pour ses travaux dans le cadre de l'ELA. L'annonce ne surprend pas beaucoup les personnes qui connaissent bien le programme ELA et la contribution remarquable qu'y a apportée le D[r] Schindler, mais elle n'en n'est pas moins bien accueillie. Pour un grand nombre d'entre nous, ce programme tout à fait unique en son genre est à l'avant-garde des efforts globaux déployés pour comprendre et protéger les écosystèmes d'eaux douces. L'annonce arrive quand même à point nommé, coïncidant avec un effort important de renforcement du mandat de l'ELA et de mise en place de la base de financement nécessaire pour renouveler et relancer le programme.

Le Prix d'hydrologie de Stockholm est le dernier exemple de la reconnaissance internationale à laquelle le programme ELA et le D[r] Schindler ont eu droit. Les analyses indépendantes ont invariablement été des plus élogieuses à l'endroit du pro-

The ELA owes its existence to an unusual set of circumstances that occurred in the 1960's. The threat of environmental degradation was beginning to be recognized. On October 7, 1964, the International Joint Commission (IJC) was asked by the governments of Canada and the United States to assess the state of pollution in the lower Great Lakes. In its interim report of December 1965, the Commission reported significant eutrophication in the lower Great Lakes. The Commission also advised governments of the need to support the research and related activities that would be required for the abatement and control of pollution of the Great Lakes. The primary focus was on cultural eutrophication and on ways of controlling this phenomenon through reduction of nutrient inputs to the lakes.

Dr. F. R. Hayes term as chairman of the FRBC extended from 1964 until 1969 and under his direction the Board responded in a major way to the IJC recommendations. Dr. Hayes brought an ecological perspective to the Board, he favoured increased linkages with the academic community, he shifted more of the work of the Board to freshwater where the major pollution problems were developing and he believed in the use of experimental approaches to clarify important cause–effect linkages. He and his deputy chairman, Dr. W. R. Martin, brought Dr. W. E. Johnson to Ottawa to develop a strategy paper to assist the Board in meeting the research needs identified during the lower lakes reference study. Dr. W. M. Sprules with the Department of Fisheries and Forestry and also a member of the FRBC served with the IJC's investigative boards on Lake Erie and on Lake Ontario and the St. Lawrence. Somewhere within that talented group of people, the concept of an ELA was born. The concept itself was both elegant and simple.

To safeguard the Great Lakes and other freshwater resources the originators saw a need to designate an area where critical whole-lake experiments could be carried out. Only in this way could promising hypotheses for protecting and restoring freshwater lakes be tested and evaluated. This critical step was necessary in order to be able to link what could be learned in the laboratory to larger freshwater ecosystems. The program began in the late 1960's as a major project of the newly created Freshwater Institute. An area southeast of Kenora, Ontario, containing 46 small lakes was set aside for research purposes through agreements involving forestry companies as well as the governments of Canada and Ontario.

Dr. Johnson, the first Director of the Freshwater Institute, was given full authority to recruit the strongest possible team to tackle freshwater pollution problems, and the ELA was always very high on his list of priorities. Dr. Johnson recruited Dr. J. R. Vallentyne to lead the new eutrophication section and the two of them were soon recruiting talent from around the world. It was a time of excitement and the sense of adventure and empowerment was infectious. Recruits knew that they were privileged to be able to participate in something that was important. A special ingredient was the unity of purpose that existed within the organization: the FRBC was providing a wonderful opportunity for those who wanted to be part of an undeclared and undefined conspiracy to save the planet. As grandiose and as unrealistic as this view of the future may have been, it did provide enough momentum to carry over into the very different climate that existed after the FRBC was absorbed within the public service.

My first meeting with David Schindler was in 1967 in Madison, Wisconsin, at an international symposium on eutrophication. I had been instructed by my supervisor, Dr. Vallentyne, to try to convince Dr. Schindler, who was then teaching at Trent

gramme, et le Dr Schindler a lui-même été lauréat de nombreux prix et médailles d'associations scientifiques. La presse populaire et le cinéma, la télévision et la radio, et notamment le *Newsweek*, le *U.S. News and World Report*, le *New York Times*, le *Washington Post*, le *Globe and Mail* de Toronto, le *Star* de Toronto, le *Citizen* d'Ottawa, et la *Free Press* de Winnipeg, ont également traité de l'ELA.

L'ELA doit son existence à un curieux concours de circonstances remontant aux années 1960, à l'époque où la menace de dégradation de l'environnement commence à préoccuper. Le 7 octobre 1964, les gouvernements du Canada et des États-Unis chargent la Commission mixte internationale (CMI) d'évaluer l'état de pollution des Grands Lacs inférieurs. Dans son rapport provisoire de décembre 1965, la Commission fait état d'une importante eutrophisation des Grands Lacs inférieurs. Elle fait également valoir aux gouvernements la nécessité de soutenir la recherche et les activités connexes qu'il faut pour réduire et limiter la pollution des Grands Lacs. Elle insiste surtout sur l'eutrophisation due aux cultures et sur les moyens de réprimer ce phénomène par la réduction des apports nutritifs dans les lacs.

Sous la direction du Dr F. R. Hayes, dont le mandant à la présidence de l'ORPC est alors prolongé de 1964 à 1969, l'Office ne ménage rien pour donner suite aux recommendations de la CMI. Le Dr Hayes donne à l'Office une perspective écologique, favorise le resserrement des liens avec les milieux universitaires, réoriente les travaux de l'Office vers les eaux douces où se manifestent les grands problèmes de pollution, et préconise le recours à des approches expérimentales pour expliquer d'importantes relations de cause à effet. Avec son vice-président, le Dr W. R. Martin, il fait venir à Ottawa le Dr W. E. Johnson, qu'il charge de préparer un document de stratégie pour aider l'Office à répondre aux besoins de recherche définis à l'occasion de l'étude sur les lacs inférieurs. Le Dr W. M. Sprules, du ministère des Pêches et Forêts et aussi membre de l'ORPC, siège aux commissions d'enquête de la CMI sur le lac Érié et sur le lac Ontario et le Saint-Laurent. C'est au sein de ce groupe extrêmement compétent que naît le concept de l'ELA. Le concept même manque ni d'élégance ni de simplicité.

Pour protéger les Grands Lacs et les autres ressources en eaux douces, les créateurs jugent nécessaire de désigner une zone où l'on pourrait mener des expériences critiques sur des lacs entiers. C'est le seul moyen de vérifier et d'évaluer des hypothèses prometteuses pour la protection et la restauration des lacs d'eaux douces. C'est une étape critique de l'établissement d'un lien entre les constatations en laboratoire et les grands écosystèmes d'eaux douces. Le programme s'enclenche à la fin des années 1960 en tant que grand projet du nouvel Institut des eaux douces. Une région située au sud-ouest de Kenora (Ontario), renfermant 46 petits lacs, est alors isolée pour fins de recherche en vertu d'accords mettant en cause les sociétés forestières ainsi que les gouvernements du Canada et de l'Ontario.

Le Dr Johnson, premier directeur de l'Institut des eaux douces, se voit donner carte blanche pour le recrutement de l'équipe la plus énergique possible pour attaquer les problèmes de pollution des eaux douces, et l'ELA a toujours été en bonne place sur sa liste de priorités. Le Dr Johnson recrute alors le Dr J. R. Vallentyne, à qui il confie la nouvelle section d'eutrophisation, et ils se mettent ensemble à la recherche des plus grands noms dans le monde entier. C'est une époque d'euphorie et l'esprit d'aventure et le sentiment du pouvoir est contagieux. Les recrues sont conscientes du privilège qu'elles ont de pouvoir participer à quelque chose d'important. Un ingrédient spé-

University, to join the Freshwater Institute. On my return, I wrote a long letter to Dr. Schindler in which I went to great lengths to outline the potential and opportunities that would be available at the Freshwater Institute. I also included an invitation to come to the Torch River country in northern Saskatchewan for the annual moose hunt that had its headquarters at the Hamilton farm. The moose hunt may have been the clincher and it did become an annual event that also provided ample time to explore ideas and to design future research strategies and research activities.

The ELA was a refuge where grandiose ideas could be tested and where there was enough freedom and flexibility to begin addressing emerging pollution threats long before they were officially recognized. The whole-lake acidification experiments that were initiated years before the issue reached Ottawa or the media are a good example of how this flexibility allowed the program to be well ahead of the crowd.

Many talented people, and unique circumstances and events, have contributed to the success of ELA. The vision and enthusiasm of the original founders at FRBC gave the program a remarkable send-off, and Dr. Schindler's dynamic and dedicated leadership was the key to the continuing success of the program. The *Journal* (formally the *Journal of the Fisheries Research Board of Canada* and now the *Canadian Journal of Fisheries and Aquatic Sciences*) has always been one of the program's major voices to the world; its editors' enthusiastic support of the program is reflected by the unprecedented number of issues dedicated to ELA results (JFRBC 28(2): 123–301; 30(10): 1409–1552; CJFAS 37(3): 311–558; 44 (Suppl. 1): 3–274). Dr. Schindler's sectional and divisional supervisors (as if anyone really supervised David) from Dr. Vallentyne to his most recent supervisor, Dr. G. D. Koshinsky, have always recognized the unique importance of the program and have gone out of their way to lend support. Other regional managers have, usually, shared this enthusiasm for the program and there has always been a nucleus of key people in Ottawa who could be counted upon for help.

Wally Broecker, John Cherry, Eville Gorham, Gene Likens, and Gordon Robinson are illustrative of the many academic leaders who have lent their support to the program. The ELA program has always been an attractive place for graduate students, and at last count, over 40 people have written their Masters or Ph.D. thesis on research carried out at the ELA site. Support of key personnel in other government departments has always been important — even essential. Illustrative examples include Jim Bruce, Tog Jackson, and Doug Whelpdale in Environment Canada, Peter Dillon in the Ontario Ministry of the Environment, and Andy Lawrie of the Ontario Ministry of Natural Resources. They have all played key roles as did Tom Brydges, first with the Ontario Ministry of the Environment and later with Environment Canada.

It was not only research planners and practitioners who played vital roles in the success of the program. Bernie Bjornson was often called upon to purchase critical equipment — usually under impossible time constraints. Eric Marshall always provided excellent library services to the ELA team. Cliff Jones and Willie Burton were almost honorary members of the ELA team.

Without doubt, ELA has been Canada's freshwater research flagship — not in terms of dollars spent but in terms of knowledge gained. David Schindler and the dedicated individuals who have made up the ELA team have a right to feel proud of their contributions to our understanding of aquatic ecosystems. The

cial est la communauté d'objectifs qui caractérise l'organisation : l'ORPC offre une merveilleuse occasion pour ceux qui veulent être d'une conspiration non déclarée et non définie pour sauver la planète. Pour grandiose et irréaliste que cette vision de l'avenir puisse alors paraître, elle imprime néanmoins un élan suffisant pour déborder dans le climat très différent qui règne après l'intégration de l'ORPC dans la fonction publique.

J'ai rencontré David Schindler la première fois en 1967 à Madison (Wisconsin), lors d'un symposium international sur l'eutrophisation. Mon superviseur, le Dr Vallentyne, m'avait charge de tâcher de convaincre le Dr Schindler, alors professeur à l'Université Trent, de se joindre à l'Institut des eaux douces. À mon retour, j'ai adressé au Dr Schindler une longue lettre dans laquelle je lui exposais par le détail les possibilités et les perspectives qu'ouvrirait l'Institut des eaux douces. J'y joignais une invitation à venir dans le coin de la rivière Torch dans le nord de la Saskatchewan pour la chasse annuelle à l'orignal, dont le point de ralliement était la ferme Hamilton. La chasse à l'orignal a peut-être été l'argument décisif, et elle est devenue un événement annuel qui a donné également toutes les occasions voulues d'explorer des idées et de concevoir des stratégies et des activités de recherche pour l'avenir.

L'ELA était un refuge où les idées grandioses pouvaient être mises à l'essai, où chacun disposait de toutes les libertés et de toute la souplesse pour commencer à réprimer les nouvelles menaces de la pollution bien avant qu'elles ne soient officiellement reconnues. Les expériences d'acidification sur lac entier qui avaient été lancées des années avant qu'Ottawa ou les médias ne s'intéressent à la question témoignent bien de la façon dont cette souplesse a permis au programme de distancer le peloton.

L'ELA doit ses succès à de nombreuses personnes d'une très grande compétence, et au caractère tout à fait particulier des circonstances et des événements. La vision et l'enthousiasme des premiers fondateurs à l'ORPC ont donné un remarquable coup d'envoi au programme, et le leadership dynamique et dévoué du Dr Schindler a été la clé des succès constant qu'a remportés le programme. Le *Journal* (anciennement le *Journal de l'office des recherches sur les pêcheries du Canada* et désormais le *Journal canadien des sciences halieutiques et aquatiques*) a toujours été l'un des grands moyens d'ouverture sur le monde pour le programme; l'appui enthousiasme de ses rédacteurs pour le programme se voit dans le nombre sans précédent de numéros consacrés aux résultats de l'ELA (JORPC 28(2): 123–301; 30(10): 1409–1552; JCSHA 37(3): 311–558; 44 (Suppl. 1): 3–274). Les superviseurs de section et de division du Dr Schindler (comme s'il avait besoin d'être supervisé), depuis le Dr Vallentyne jusqu'au plus récent, le Dr G. D. Koshinsky, ont toujours reconnu l'importance particulière du programme et n'ont rien ménagé pour l'appuyer. D'autres gestionnaires régionaux ont, habituellement, partagé cet enthousiasme pour le programme et il y a toujours eu à Ottawa un noyau de personnes clés sur qui compter.

Wally Broecker, John Cherry, Eville Gorham, Gene Likens et Gordon Robinson ne sont que quelques-uns des nombreux universitaires de renom qui ont prêté leur appui au programme. Le programme ELA a toujours séduit les étudiants de deuxième et de troisième cycle et, selon le dernier dénombrement, plus de 40 personnes ont fondé leur thèse de maîtrise ou de doctorat sur des recherches effectuées dans la région ELA. L'appui de personnel clé d'autres ministères a toujours été important — voire essentiel. Qu'il suffise de mentionner Jim Bruce, Tog Jackson et Doug Whelpdale d'Environnement Canada, Peter

program has also had a remarkable record of transferring that knowledge in ways that affect public opinion and public policy. The readiness to become involved and to speak up on issues of concern to the public was essential if the program was to lead to public action to protect freshwater ecosystems and freshwater habitats.

For two decades, Dr. Schindler had been able to demonstrate how a program that was built on the "bottom-up" approach to testing scientific ideas and hypothesis rather than a "top-down" mode of executing managerial directives could set the standard in terms of both excellence and long-term relevance.

In 1989, he joined the University of Alberta as the Killam professor of ecology; however, David continues to be an active participant in ELA research.

The Department of Fisheries and Oceans has had a commendable record of supporting the ELA program. The challenge in the years to come is to ensure that the mandate and the resources are adequate for the job that lies ahead. Given the environmental problems of the 1990's, it is a job that has really just begun.

Andrew L. Hamilton
Director, Special Projects
Rawson Academy of Aquatic Science
1 Nicholas Street
Ottawa, Ont. K1N 7B7

Dillon du ministère de l'Environnement de l'Ontario, et Andy Lawrie du ministère des Richesses naturelles de l'Ontario. Ils ont tous joué des rôles clés, tout comme d'ailleurs Tom Brydges, d'abord du ministère de l'Environnement de l'Ontario, puis d'Environnement Canada.

Et ce ne sont pas seulement les planificateurs de la recherche et les chercheurs mêmes qui ont joué des rôles cruciaux dans le succès du programme. Bernie Bjornson a souvent été mis à contribution pour l'achat du matériel critique — habituellement dans des délais impossibles. Eric Marshall a toujours assuré d'excellents services de bibliothèque à l'équipe de l'ELA. Cliff Jones et Willie Burton étaient presque des membres honoraires de l'équipe de l'ELA.

Sans contredit, l'ELA a été le navire amiral de la recherche sur les eaux douces du Canada — non pas par les dollars dépensés, mais par les connaissances acquises. David Schindler et les personnes qui se sont dévouées au sein de l'équipe de l'ELA ont le droit d'être fiers de leur contribution à notre connaissance des écosystèmes aquatiques. Le programme a aussi un retentissant palmarès de transfert de ses connaissances selon des modalités qui infléchissent l'opinion publique et la politique gouvernementale. Il était essentiel d'être prêt à s'impliquer et à élever la voix sur les questions préoccupant le public afin que le programme débouche sur une action publique destinée à protéger les écosytèmes et les habitats d'eaux douces.

Pendant deux décennies, le D[r] Schindler a su démontrer comment un programme fondé sur une approche « de la base vers le sommet » pour la vérification d'idées et d'hypothèses scientifiques plutôt que sur le mode « du sommet vers le bas » caractérisé par la mise à exécution de directives de gestion peut définir la norme d'excellence et de pertinence à long terme.

En 1989, il se joint à l'Université de l'Alberta, en qualité de professeur Killam d'écologie. Toutefois, David continue de participer activement aux projets de recherche dans le cadre du programme ELA.

Le ministère des Pêches et des Océans a accordé un appui louable au programme ELA. Le défi des années à venir consistera à assurer le mandat et les ressources qu'il faut pour le travail qui reste à faire. Compte tenu des problèmes écologiques des années 1990, c'est un travail qui en réalité commence à peine.

Andrew L. Hamilton
Directeur, projects spéciaux
Académie Rawson des sciences de l'eau
1, rue Nicholas
Ottawa (Ontario) K1N 7B7

Controls on Domoic Acid Production by the Diatom *Nitzschia pungens* f. *multiseries* in Culture: Nutrients and Irradiance[1]

S. S. Bates[2]

Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6, Canada

A. S. W. de Freitas and J. E. Milley

Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford St., Halifax, N.S. B3H 3Z1, Canada

R. Pocklington

Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada

M. A. Quilliam

Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford St., Halifax, N.S. B3H 3Z1, Canada

and J. C. Smith and J. Worms

Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6, Canada

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Nitzschia pungens f. *multiseries* (clone NPARL) was grown in nonaxenic batch culture under a range of growth conditions. Domoic acid (DA) was not detected during exponential growth, but production promptly started at a rate of approximately 1 pg DA·cell^{-1}·d^{-1} at the onset of the stationary phase, in this case induced by silicate limitation. Cellular DA reached a maximum of 7 pg·cell^{-1}; thereafter, DA production continued at the same rate, with cellular levels remaining relatively constant due to concurrent release of DA into the culture medium. DA production ceased in the absence of nitrogen during the stationary phase, but resumed when nitrate was added back to the medium. Low irradiance slowed the division rate and consequently delayed the attainment of the stationary phase, but DA production rates were comparable with the control once stationary phase was reached. Cells during the dark period of a light–dark cycle, or placed into darkness, or in the presence of the photosynthetic inhibitor DCMU promptly ceased DA production. We conclude that at least three conditions are required for DA production by clone NPARL: cessation of cell division, availability of nitrogen during the stationary phase, and the presence of light. Growth in medium f/2 fulfils these requirements.

On a effectué la culture discontinue non axénique de la diatomée *Nitzschia pungens* f. *multiseries* (clone NPARL) dans diverses conditions de croissance. On n'a pas relevé la présence d'acide domoïque (AD) pendant la phase de croissance exponentielle, mais la production a commencé sans tarder au taux d'environ 1 pg AD·cellule^{-1}·jour^{-1} dès le début de la phase de croissance stationnaire, provoquée dans ce cas-ci par la pénurie de silicate. La concentration cellulaire d'AD a atteint un maximum de 7 pg·cellule^{-1}; par la suite, la production d'AD s'est poursuivie au même taux et les teneurs cellulaires sont demeurées relativement constantes étant donné la libération simultanée d'AD dans le milieu de culture. La production d'AD a cessé en l'absence d'azote pendant la phase stationnaire, mais a recommencé après ajout de nitrate au milieu de culture. Un faible éclairement a ralenti le taux de division des cellules et par conséquent la réalisation de la phase stationnaire, mais les taux de production d'AD étaient comparables à ceux obtenus dans le cas de la culture témoin une fois la phase stationnaire atteinte. Des cellules placées à l'obscurité, exposées à la phase obscure d'un cycle jour–nuit, ou mises en présence d'un inhibiteur de la photosynthèse, le DCMU, ont rapidement cessé toute production d'AD. Les auteurs formulent la conclusion que le clone NPARL requiert au moins trois conditions pour la production d'AD, soit l'absence de la division cellulaire, la disponibilité d'azote pendant la phase stationnaire et la présence de lumière. La croissance en milieu de culture f/2 satisfait à ces trois conditions.

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The pennate diatom *Nitzschia pungens* forma *multiseries* (Hasle 1965) has bloomed for at least the past four autumns in the Cardigan Bay region of eastern Prince Edward Island (P.E.I.), Canada (Smith et al. 1989, 1990a, 1990b). This diatom was the primary source of the neurotoxin domoic acid (Wright et al. 1989; Bates et al. 1989), which contaminated cultured blue mussels (*Mytilus edulis*) and resulted in 107 cases of human poisoning and three deaths in 1987 (Perl et al. 1990). Amnesic shellfish poisoning (ASP) results from

[1]Contribution No. NRCC 31959.
[2]Author to whom correspondence should be addressed.

the consumption of domoic-acid-containing shellfish (Todd 1990). The 1987 ASP episode was a serious threat to the molluscan aquaculture industry, but the establishment of a monitoring program by the Department of Fisheries and Oceans has provided an early warning of toxic events that has protected the industry and consumers of molluscan shellfish since 1988 (Bourque and Cormier 1989).

Nitzschia pungens f. *multiseries* from Cardigan Bay was first isolated into unialgal culture by Subba Rao et al. (1988) who demonstrated the de novo production of domoic acid by nonaxenic cultures of this diatom. Further work established that this diatom was the primary source of domoic acid in cultured mussels (Bates et al. 1989). Other organisms, however, may be implicated in domoic acid production, as this toxin has been detected in *Nitzschia pseudodelicatissima* (Martin et al. 1990) as well as in the absence of *N. pungens* f. *multiseries* (Smith et al. 1990b). Three isolates of *N. pungens* f. *multiseries* from Cardigan Bay produced domoic acid under comparable culture conditions (Bates et al. 1989; Subba Rao et al. 1990). Recent isolates of *N. pungens* f. *multiseries* from Galveston Channel, Texas, have also been shown to produce domoic acid (Fryxell et al. 1990). *Nitzschia pungens* has been reported from coastal waters around the world (Hasle 1965; Furnas 1982; Gonzalez-Rodriguez et al. 1985; Forbes and Denman 1991), but its ability to produce domoic acid in these waters has not been studied.

The factors influencing the production of domoic acid are not yet well understood. Here we document the production and release of domoic acid in culture by *N. pungens* f. *multiseries*. We show that domoic acid is produced only during the stationary phase of batch culture and that irradiance and the presence of extracellular nitrogen are essential for domoic acid production.

Materials and Methods

Organism and Culture Conditions

Nitzschia pungens f. *multiseries* (clone NPARL) was isolated from a sample collected in Cardigan Bay, P.E.I. (Bates et al. 1989). The unialgal isolate was nonaxenic, but sterile technique was used throughout the study. Until very recently, repeated attempts to render the culture axenic have failed, but studies with axenic cultures are now in progress. Cells were maintained in medium f/2 (Guillard and Ryther 1962) made with filtered seawater. Cultures were normally grown in borosilicate flasks, but polycarbonate flasks were used when control of the silica concentration was specifically required. The temperature, irradiance, and nutrient conditions are described with each experiment. Irradiance was provided by a bank of Cool-White fluorescence lamps and was measured with a LI-COR quantum meter (model LI-185B) fitted with a 4π sensor. Cell numbers were obtained by optical microscope counts of two to four replicate 5-μL aliquots of culture. The concentration of major nutrients was determined with a Technicon autoanalyzer.

Sample Preparation for Domoic Acid Analysis

Three methods of harvesting *N. pungens* cultures were tested to determine the best procedure for differentiating between cellular and extracellular (that found in the culture medium after removing the *N. pungens* cells) domoic acid.

(1) Concentration using polycarbonate filters

Cells from duplicate 50-mL aliquots of culture were concentrated down to approximately 2 mL (ensuring that cells on the filter never went dry) by vacuum filtration (<10 kPa) onto a polycarbonate-membrane filter (Nuclepore; 47-mm diameter; 3-μm pore size). Cells were then rinsed with three 10-mL volumes of filtered seawater, quantitatively removed from the filter using a pipette, and made to 10 mL with seawater. These cells and the filtrate were separately frozen for subsequent analysis.

(2) Filtration onto glass-fibre filters

Cells from duplicate 50-mL aliquots of culture were collected by vacuum filtration (<10 kPa) onto a glass-fibre filter (Whatman type GF/F; 47-mm diameter). Again, cells on the filter were never allowed to go dry. The filter and the filtrate were frozen for subsequent analysis.

(3) Centrifugation

Duplicate 10-mL aliquots of culture were centrifuged in conical tubes for 5 min at approximately 900 × *g*, then 5 mL of supernatant from each aliquot was removed for domoic acid analysis, and the remaining material was again centrifuged for 5 min at 900 × *g*. All but approximately 0.2 mL of supernatant was removed, the volume was made to 10 mL with filtered seawater, and the sample was then frozen.

Prior to domoic acid analysis, *N. pungens* cells, concentrated by filtration onto a membrane filter or by centrifugation, were sonicated for 1 min at 100 W using a 1-cm-diameter probe (Braun-sonic 1510) to release the cellular domoic acid. Cell debris was then removed by filtration through a Millex-HV 0.45-μm disposable filter (Millipore Corp.). Glass-fibre filters containing the cells were sonicated for 3 min in 9 mL of seawater, and the debris was removed by centrifugation at 1000 × *g* for 10 min. The supernatant was made to 10 mL with seawater and then filtered through a 0.45-μm disposable filter prior to domoic acid analysis.

Domoic acid was analyzed by a high-sensitivity (0.5 ng·mL^{-1} detection limit) 9-fluorenylmethoxycarbonyl chloride (FMOC) precolumn derivatization method for amino acids followed by reversed-phase HPLC with fluorescence detection (Pocklington et al. 1990).

The domoic acid concentration in the cells (picograms per cell) was calculated by dividing the concentration of domoic acid in the cellular extract made to 10 mL with seawater (micrograms per millilitre) by the cell concentration (cells per millilitre). Domoic acid produced by the "whole culture" (i.e. cells sonicated with the growth medium) was obtained after sonication, as above. Cellular domoic acid from the "whole culture" (picograms per cell) was calculated by attributing the total domoic acid producted in the culture to the cells.

Domoic Acid Secretion Experiment

A time-course experiment was carried out to monitor the change in cellular and extracellular domoic acid in a batch culture of *N. pungens*. Cells were grown (15°C; 100 μE·m^{-2}·s^{-1}; in a borosilicate carboy containing 16 L of f/2 medium (214 μM Si). An orbital shaker (90 rpm agitation) and aeration with filter-sterilized air kept the culture well mixed. Cellular domoic acid was measured in cells harvested onto polycarbonate filters, as above. Extracellular domoic acid was measured in the culture medium after the cells were removed by filtration.

Irradiance Experiments

The timing and magnitude of domoic acid production were determined on *N. pungens* cultures grown at two irradiance

levels (145 and 45 $\mu E \cdot m^{-2} \cdot s^{-1}$; 10 h light : 14 h dark) attenuated by neutral density screens. Our preliminary experiments indicate that photosynthesis is saturated at an irradiance level of 150–200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Cultures were grown, in triplicate, at 10°C in flasks containing 300 mL of f/2 medium (214 μM Si). The flasks were placed on an orbital shaker table (100 rpm agitation) and the position of the flasks was rotated daily to account for any uneven distribution of irradiance level.

The effect of darkness on domoic acid production by *N. pungens* was determined by placing a stationary phase culture into darkness for 7 d. Another experiment examined the production of domoic acid during light–dark cycles in an early stationary phase culture (15°C; 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; 16 h light : 8 h dark).

Photosynthetic Inhibitor Experiment

An experiment was carried out to determine the effects of the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on domoic acid production by the "whole culture". *Nitzschia pungens* was grown (15°C; 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; 16 h light : 8 h dark) in duplicate flasks containing 250 mL of f/2 medium (214 μM Si). At the midexponential phase, DCMU (10^{-5} M final concentration made from a 10^{-3} M ethanolic stock solution) was added to the experimental flasks and ethanol (1% final concentration) to the control flask.

Nutrient Experiments

One experiment examined the production of domoic acid and the final cell yield attained in stationary phase as a function of initial silicate concentration. *Nitzschia pungens* was grown (15°C; 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; 16 h light : 8 h dark) in polycarbonate flasks containing initial silicate concentrations of 5, 10, 30, 55, and 105 μM. Domoic acid in the "whole culture" and in the filtrate was determined on cultures fractionated using polycarbonate-membrane filters during the stationary phase, and cellular domoic acid was determined by difference.

Other experiments were carried out (15°C; 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; 16 h light : 8 h dark) to examine the effects of the presence or absence of major nutrients on domoic acid production in stationary phase cultures of *N. pungens*. Cells from a late-exponential phase culture were gently (<10 kPa) concentrated onto a membrane filter and resuspended into each of three Fernbach flasks containing 1.0 L of either fresh f/2 medium with no nitrate, silicate, or phosphate and into a control flask containing the full complement of f/2 nutrients.

A final set of experiments examined the effects of nitrate, in particular, on domoic acid production. Duplicate cultures of *N. pungens* were grown (17°C; 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; 16 h light : 8 h dark) in flasks containing 300 mL of f/2 medium with nitrate-N initially at approximately 1 mM, 0.05 mM, or with no nitrate. On day 14 (9 d after the beginning of the stationary phase), nitrate became undetectable in the culture grown initially with 0.05 mM nitrate-N. At that point, nitrate-N (approximately 1 mM final concentration) was added to the culture to determine if nitrogen addition stimulated domoic acid production.

Results

Cell Harvesting

Separating cellular from extracellular domoic acid using polycarbonate-membrane filtration and by centrifugation gave

TABLE 1. Comparison of three methods for harvesting *N. pungens* cells for domoic acid analysis: A = concentration onto a Nuclepore filter; B = centrifugation; C = collection onto a glass-fibre filter. Domoic acid concentrations (n = 2) are given for cells and for the filtrate or centrifugate, measured on the indicated days during the stationary phase in batch culture. Total concentrations (addition of domoic acid in cells and domoic acid in filtrate) are compared with the domoic acid measured in the "whole culture" (i.e. cells sonicated with the growth medium), indicated as method D. The domoic acid in the column labelled "Cells" was obtained by multiplying the cell concentration by the concentration of cellular domoic acid.

Day	Method	Cell number ($cells \cdot mL^{-1}$)	Domoic acid ($pg \cdot cell^{-1}$)	Domoic acid ($\mu g \cdot mL^{-1}$)		
				Cells	Filtrate	Total
12	A	190 250	6.8	1.30	0.80	2.10
	B	190 250	6.5	1.24	0.80	2.04
	C	190 250	2.0	0.38	0.76	1.14
	D	190 250	—	—	—	1.94
	A	191 000	5.7	1.10	1.65	2.75
	B	191 000	5.3	1.00	1.70	2.70
	C	191 000	2.8	0.54	1.70	2.24
19	D	191 000	—	—	—	2.80
	A	190 000	4.2	0.80	2.00	2.80
	B	190 000	5.3	1.00	2.20	3.20
	C	190 000	2.9	0.50	2.20	2.70
27	D	190 000	—	—	—	2.80
	A	189 850	4.7	0.90	2.70	3.60
	B	189 850	4.4	0.80	2.70	3.53
	C	189 850	3.1	0.50	2.60	3.10
34	D	189 850	—	—	—	3.40

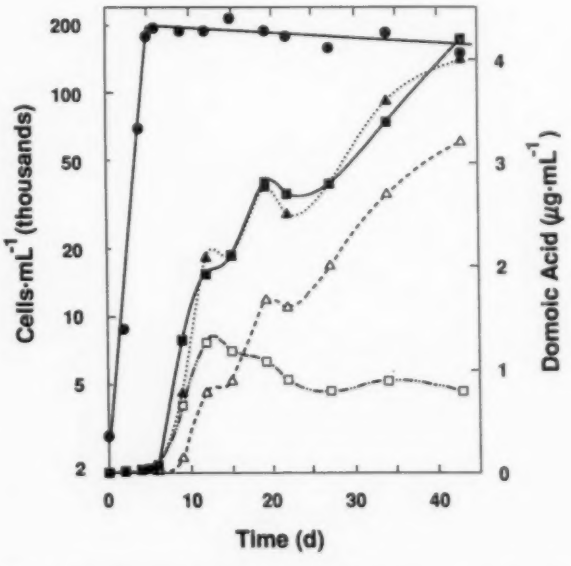

FIG. 1. Growth and domoic acid production by *N. pungens* f. *multiseries* in batch culture. Cell number (solid circles); domoic acid in "whole culture" (solid squares), in cells plus filtrate (solid triangles), in filtrate (open triangles), and in cells (open squares).

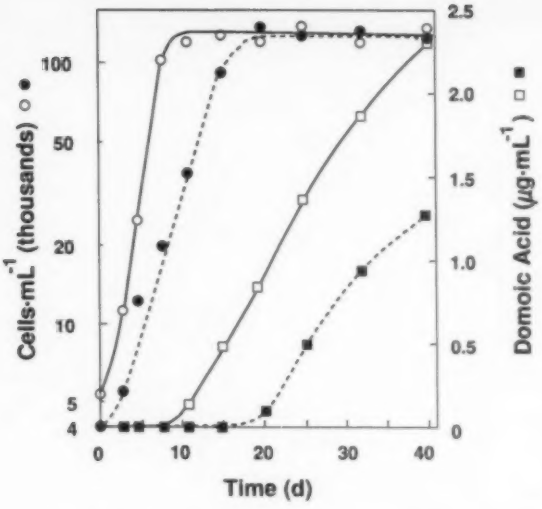

FIG. 2. Cell number (circles) and domoic acid concentration in the "whole culture" (squares) of *N. pungens* f. *multiseries* grown at two irradiance levels: 45 μE·m^{-2}·s^{-1} (solid symbols) and 145 μE·m^{-2}·s^{-1} (open symbols).

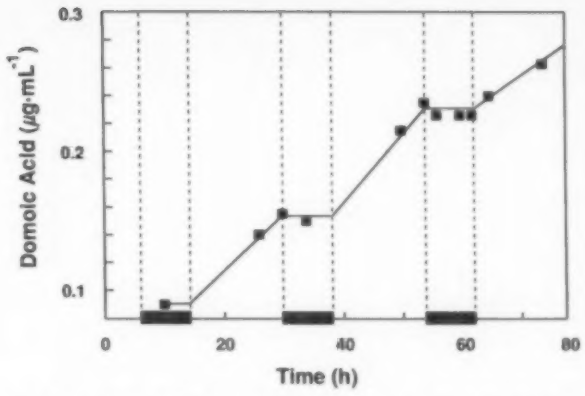

FIG. 3. Domoic acid concentration in the "whole culture" of *N. pungens* f. *multiseries* during early stationary phase in a batch culture on a 16 h light : 8 h dark cycle. The dark periods are shown by black bars; the line drawn through the points is idealized.

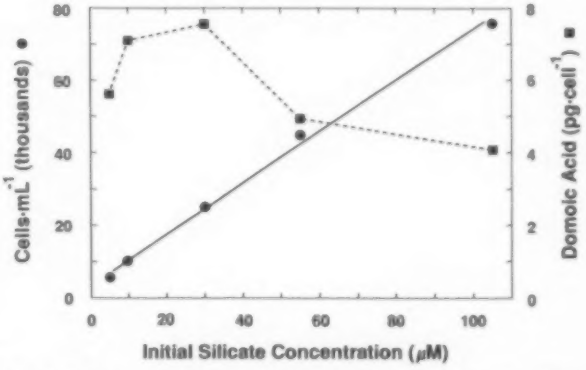

FIG. 4. Cell number (circles) and cellular domoic acid concentration (squares) for *N. pungens* f. *multiseries* grown at different initial silicate concentrations in batch culture. All cultures were harvested on the same day, so each culture remained in stationary phase for a different period of time.

comparable results for samples taken during the stationary phase (Table 1). The total of cellular plus extracellular domoic acid compared favorably with that in the "whole culture" (i.e. cells sonicated with the growth medium), indicating that the methods used for differentiating cellular from extracellular domoic acid were quantitatively suitable. In contrast, concentrating the cells onto a glass-fibre filter gave low values for cellular domoic acid (Table 1). This may have been due to a poor extraction efficiency or to loss of domoic acid by adsorption onto the glass fibres when they were removed by centrifugation. The latter is not likely because the domoic acid concentration in the filtrate from glass-fibre filtration was similar to that from polycarbonate-membrane filtration or from centrifugation (Table 1). Given the greater variability with glass-fibre filters, harvesting by concentrating cells with a membrane filter was the method used in all subsequent experiments in which cellular and extracellular domoic acid were differentiated.

Domoic Acid Secretion Experiment

As in previous experiments (Bates et al. 1989), domoic acid was produced only during the stationary phase in batch culture (Fig. 1). Cellular domoic acid reached a maximum (7 pg·cell^{-1}) about 7 d after the beginning of the stationary phase, and slowly declined thereafter. Extracellular domoic acid in the filtrate lagged the appearance of cellular domoic acid by about 3 d and continued to increase with time in the growth medium, eventually exceeding the quantity found in the cells. The concentration of cellular plus extracellular domoic acid equalled the concentration of domoic acid in the "whole culture", indicating that the results properly account for the domoic acid in the two fractions.

Irradiance Experiments

Incubation at a high irradiance level resulted in a significantly faster ($p = 0.05$) growth rate (0.64 divisions·d^{-1}) than that observed at a lower irradiance level (0.35 divisions·d^{-1}), as expected (Fig. 2). The cell yields attained during the stationary phase were similar at both irradiance levels. Because of the faster growth rate, however, the stationary phase was reached about 10 d earlier in the high- than in the low-irradiance culture. As in the above experiment, domoic acid in the "whole culture" was detected only after the beginning of the stationary phase. For the high-irradiance culture, this was about 10 d earlier than for the low-irradiance culture. The initial rates of domoic acid production were not significantly different ($p = 0.01$) at the two irradiance levels (Fig. 2).

Domoic acid production ceased when a stationary phase culture (5 d after the beginning of the stationary phase) was placed into darkness. Likewise, there was no production of domoic acid during the dark period when *N. pungens* was grown with a 16 h light : 8 h dark cycle (Fig. 3). The production of domoic acid stopped rapidly (within 2h) after the transition from the light to the dark period, and promptly resumed after the beginning of the light cycle.

Nutrient Experiments

A positive linear relationship ($r = 0.998$) was found between the initial silicate concentration added to the f/2 medium and

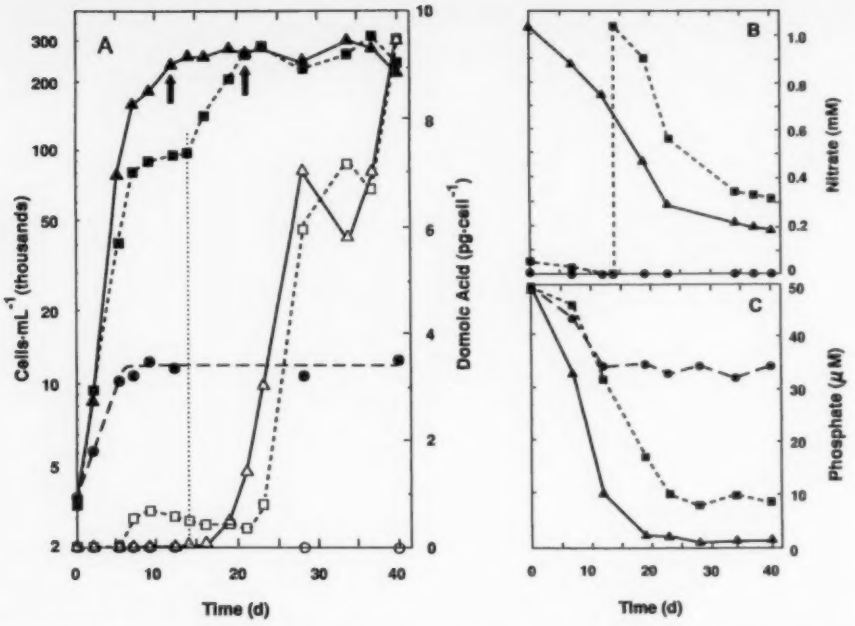

FIG. 5. (A) Effect of nitrate addition on cell number (solid symbols) and domoic acid concentration in the "whole culture" (open symbols) of *N. pungens* f. *multiseries*. (B) Concentration of nitrate and (C) concentration of phosphate in the same batch cultures as shown in Fig. 5A. Culture grown in the presence of an initial nitrate-N concentration of 1 mM (triangles); culture grown with an initial nitrate-N concentration of 0.05 mM and then supplemented with 1 mM nitrate-N at the time indicated by the dotted line (squares); culture grown in the absence of nitrate (circles). The silicate concentration decreased to below 10 μM at the times indicated by the arrows in Fig. 5A.

cell yield obtained during the stationary phase in batch culture (Fig. 4). Division rates were not significantly different for each of the silicate concentrations. Cultures grown with initial silicate concentrations of 5–30 μM contained approximately 35% more domoic acid per cell at the time of harvest during the stationary phase than those grown with 55–105 μM initial silicate (Fig. 4). It should be noted that because of the length of time required to deplete the added silicate, each culture had been at stationary phase for a different duration at the time of harvest, and this could explain the differences in cellular domoic acid content, as discussed below.

Preliminary experiments showed that domoic acid production continued when silicate-depleted medium from a stationary phase culture was replaced with fresh f/2 medium containing nitrate but no phosphate or silicate. Production of domoic acid ceased, however, when the spent medium was replaced with f/2 medium containing no nitrate. This led to a more detailed examination of the effects of three nitrate concentrations on domoic acid production.

Two batch cultures were grown in f/2 medium containing limiting concentrations of nitrate (0 and 0.05 mM). A third (control) culture was grown in medium containing excess nitrate (approximately 1 mM) and a limiting concentration of silicate (185 μM). With no added nitrate, *N. pungens* cell numbers increased from an initial 3700 cells·mL^{-1} to a final 11 000 cells·mL^{-1} (approximately 1.3 doublings), and domoic acid remained undetectable during the stationary phase (Fig. 5A). When grown with an initial nitrate concentration of 0.05 mM, a cell density of approximately 90 000 cells·mL^{-1} was reached (Fig. 5A), limited by the depletion of nitrate (Fig. 5B); phosphate never became depleted in any of the cultures (Fig. 5C). A low level of domoic acid (approximately 0.4 pg·cell^{-1}) was found during the initial part of the nitrate-induced stationary phase (Fig. 5A). On the seventh day after the beginning of the nitrate-induced stationary phase, nitrate was added to the culture (dotted line in Fig. 5A) to bring the concentration to approximately 1 mM (Fig. 5B). This resulted in an immediate resumption of exponential growth, during which time the amount of cellular domoic acid on a per cell basis decreased due to dilution by the increase in cell number (Fig. 5A). A new stationary phase was then reached at a higher cell concentration (256 000 cells·mL^{-1}) when silicate became depleted (arrow in Fig. 5A). The new plateau level was similar to that attained by the control culture, also limited by silicate (arrow in Fig. 5A). On attaining this new plateau, cellular domoic acid began to rapidly increase at a rate similar to the control culture, reaching a value of approximately 10 pg·cell^{-1} on day 40 (Fig. 5A). The nitrate concentration never decreased below approximately 0.2 mM during the stationary phase (Fig. 5B) when domoic acid was being produced.

Incubations in the above experiment were carried out in borosilicate flasks. The silicate concentration in these flasks increased by a variable amount after autoclaving the medium. An experiment confirmed that autoclaving the medium in borosilicate flasks resulted in an increase in silicate concentration (Table 2). Because the silicate concentration can regulate the final cell yield reached during stationary phase, it is essential to be able to control this concentration, e.g. by using polycar-

TABLE 2. Concentration (±SD; $n = 6$) of silicate, phosphate, and nitrate in f/2 medium before and after autoclaving in borosilicate or polycarbonate flasks.

Flask type	Condition	Nutrient concentration (μM) Si	P	N
Borosilicate	Preautoclaved	207 ± 6	37.1 ± 0.6	909 ± 9
	Postautoclaved	371 ± 10	34.7 ± 2.4	951 ± 20
Polycarbonate	Preautoclaved	209 ± 5	38.4 ± 1.1	914 ± 9
	Postautoclaved	176 ± 5	36.7 ± 0.9	947 ± 13

bonate flasks. When the above nitrogen growth experiment was repeated with incubations in polycarbonate flasks, lower cell yields were attained, as expected if no silica were being leached from the flasks, but the pattern of domoic acid production after nitrate addition was the same as that described above.

Photosynthetic Inhibitor Experiment

Cell division ceased within 1 d of adding an ethanolic solution of the photosynthetic inhibitor DCMU (Fig. 6). The division rate of the control culture also slowed after addition of ethanol (1% final concentration), but the final cell yields was nevertheless greater than in the DCMU-inhibited culture (Fig. 6). Domoic acid was produced by the control culture which contained ethanol, although about 10-fold less than what was usually observed (e.g. Fig. 1). No domoic acid was produced in the culture to which DCMU was added (Fig. 6).

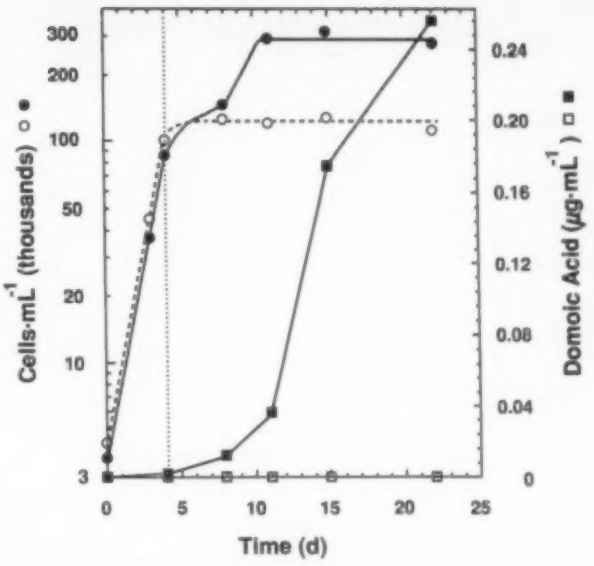

FIG. 6. Effect of addition of the photosynthetic inhibitor DCMU on cell number (circles) and domoic acid concentration in the "whole culture" (squares) of *N. pungens* f. *multiseries*. Control culture to which 1% ethanol was added (solid symbols); culture to which DCMU dissolved in 1% ethanol was added (open symbols). The additions were made at the time indicated by the dotted line.

Discussion

The events surrounding the episode of amnesic shellfish poisoning in late 1987 were unique in that they involved the production of a neurotoxin by a pennate diatom, *Nitzschia pungens* f. *multiseries*, and that the diatom bloom and toxic mussels were localized to within a narrow coastal region of eastern Prince Edward Island (Bates et al. 1988, 1989). Autumnal blooms of *N. pungens* have occurred now for the last four consecutive years (Smith et al. 1989, 1990b), and the causes of these blooms remain unclear. However, results of this study identify some of the conditions necessary for domoic acid production by *N. pungens* f. *multiseries* in laboratory culture.

The methodology we used to harvest *N. pungens* cultures (i.e. by polycarbonate-membrane filtration) provides an adequate way to partition cellular and extracellular domoic acid. This distinction is not always necessary, in which case the measurement of domoic acid in the "whole culture" is more simply carried out by sonicating the cells plus growth medium, filtering, and analyzing the resulting filtrate.

It is well known that the environmental conditions under which phytoplankton grow and the growth stage in batch culture influence cellular chemical composition (Harrison et al. 1990). It is therefore not surprising that the toxin content of phytoplankton cells also varies with growth condition and stage in batch culture. The pattern of toxin production generally followed by dinoflagellates, for example, is for cellular levels to be highest during exponential phase and to decline during stationary phase (Singh et al. 1982; Boyer et al. 1987; Boczar et al. 1988; Roszell et al 1990; Anderson et al. 1990). This type of "growth stage variability" (Anderson et al. 1990) is markedly different from the pattern we observed for domoic acid production in batch cultures of the pennate diatom *N. pungens*, in that toxin production occurred only in stationary phase and was not evident during exponential growth. Changes in the rate of secondary metabolite production reflect the cells' changing physiology during the lag, exponential, and stationary phases in batch culture. In this context, it is evident that a batch culture consists of a mixture of physiologically distinct cells, and that at any given time, even during exponential growth, some cells may be dividing at a very slow rate or not dividing at all and hence are likely to be producing domoic acid. This would be the case, in particular, for cultures in transition between the exponential and stationary phases. Our results support this, as evidenced by a very small increase in domoic acid level during this transition period (Fig. 1 and 2).

Our experiments show that a source of extracellular nitrogen, in this case in the form of nitrate, must be available in order for domoic acid to be produced during the stationary phase. Nitrogen is similarly required for the production of the paralytic shellfish poison saxitoxin by various *Alexandrium* species, although during exponential phase (Boyer et al. 1987; Anderson et al. 1990). In the case of *Alexandrium fundyense*, saxitoxin was synthesized during stationary phase, as well as exponential phase, when nitrate was in excess and phosphorus was the growth-limiting nutrient (Anderson et al. 1990). Since both saxitoxin and domoic acid are nitrogen-containing compounds, it is not surprising that their production is dependent on an available source of nitogen. This conclusion is reinforced by results which show that *N. pungens* cells deprived of nitrogen did not produce domoic acid and that addition of nitrate resulted in the resumption of toxin production, but only after cell division ceased (Fig. 5A). When nitrogen is no longer required for cell division because of growth limitation by other factors (e.g. silicate limitation in these experiments), non-dividing cells continue to assimilate extracellular nitrogen (Fig. 5B). This nitro-

gen is involved in the synthesis of amino acids (e.g. glutamate), which may then be used for domoic acid production (Laycock et al. 1989). Extracellular nitrogen, rather than previously stored nitrogenous organic compounds, thus appears to be the primary nitrogen source for domoic acid production.

There are probably many other factors that can trigger the metabolic pathway for domoic acid production, with or without impairing the cell's ability to divide. Studies are in progress to explore this question by looking at the effects of other nitrogenous nutrients, such as ammonium and urea, on domoic acid production. Such studies may help us understand results from the field which show the maintenance of relatively high levels of domoic acid per cell in apparently actively dividing bloom populations of *N. pungens* (Smith et al. 1990b).

The inability of *N. pungens* to produce a nitrogen-containing compound, domoic acid, in the absence of nitrate and its ability to produce the toxin when cell division has ceased (in these experiments due to silicate limitation) prompted us to examine the composition of the culture medium in which the organism grew. A comparison of the N:P:Si ratio in medium f/2 and in diatoms suggests that silicate rather than nitrogen or phosphorus limits the cell yield of diatoms in this medium. We thus found, as expected, that the concentration of initial silicate controlled the final cell yield obtained during the stationary phase (Fig. 4), that silicate became depleted before nitrate or phosphate, and that nitrate remained in excess in the medium during the postexponential growth phase (Fig. 5). If one wishes to vary the initial concentration of silicate and/or nitrate, then there is an N:Si ratio below which there is a changeover from silicate to nitrate limitation. In such situations, stationary phase is induced by nitrogen limitation, and domoic acid production will not be observed. An elevated cell yield in association with domoic acid production can be achieved by increasing the silicate concentration, but the nitrate concentration must be increased proportionally in order to maintain an initial N:Si ratio of at least 8:1 as found in medium f/2. Thus, our results support other studies (e.g. Anderson et al. 1990) which show that nutrient ratios are important in controlling toxin production. In a broader context, Smayda (1990) suggested that changes in nutrient ratios could be an important factor in explaining the apparent global increase in the occurrence of novel and nuisance phytoplankton blooms, although the nutrient ratio theory does not address the question of the influence on toxin production.

The finding of a higher cellular domoic acid level in cultures grown with 55 μM silicate compared with 105 μM silicate (Fig. 4) may be explained by the timing of cell harvest during the stationary phase. All of the cultures growing with the different silicate concentrations used were harvested on the same day. This resulted in each culture remaining in stationary phase for a different duration, depending on the initial silicate concentration. Cultures in the higher initial silicate media grew for a longer period of time during the exponential phase before depleting the silicate. They therefore reached the stationary phase later than the cultures grown at the lower concentrations of initial silicate and had relatively less time to produce domoic acid. The timing of sampling as well as the initial N:Si ratio in the culture medium should therefore be carefully considered in domoic acid experiments.

We observed that the silicate concentration, measured after autoclaving the growth medium, far exceeded that originally added to the medium (Table 2). It is likely that this extra silicate was released into solution from the wall of the borosilicate culture flask during autoclaving (Tarapchak et al. 1983). The amount of silicate released could vary, depending on the age of the flask, the pH of the medium during autoclaving, and the duration of autoclaving. Use of polycarbonate flasks to avoid leaching of silica from the culture vessel allows better control over the silicate concentration, particularly when studying the production of domoic acid by low cell density stationary phase cultures.

Superimposed on the nutrient-induced "growth stage variability" in batch culture is a second type of variability due to physical factors (Anderson et al. 1990). Temperature, salinity, and irradiance level influence the production of phycotoxins, either by direct effects on metabolic pathways or indirectly via their control on division rate (Ogata et al. 1989; Tracey et al. 1990; Anderson et al. 1990). These studies generally show an increase in cellular toxin level with a decrease in division rate at suboptimal temperatures or irradiance levels. In the present study with *N. pungens*, growth at 45 $\mu E \cdot m^{-2} \cdot s^{-1}$ relative to 145 $\mu E \cdot m^{-2} \cdot s^{-1}$ resulted in a decrease in the division rate, but not in the rate of domoic acid production measured 10 d later during the stationary phase (Fig. 2). Consistent with previous experiments, domoic acid synthesis did not start until the stationary phase was reached, and this was delayed due to the slower division rate at the lower irradiance level. The above irradiance and silicate experiments illustrate the importance of measuring the time course of toxin production rather than making one measurement during the stationary phase.

There may be an irradiance level below which the rate of domoic acid production decreases if photosynthetic energy is required for toxin production. At the extreme, domoic acid synthesis ceased when *N. pungens* was placed into darkness for several days and during the dark period of a light–dark cycle (Fig. 3). This indicates that there is a close coupling between photosynthetic activity and domoic acid synthesis. This is also supported by the finding that no domoic acid was produced in the presence of the photosynthetic inhibitor DCMU (Fig. 6). A similar important role of photosynthesis in the production of dinoflagellate toxins was reported by Ogata et al. (1989). Irradiance level could also have an indirect influence on domoic acid production by its effect on the uptake of nitrate. For example, the rate of nitrate uptake by phytoplankton is typically decreased at low irradiance levels (e.g. Cochlan et al. 1991), and this could limit the supply of nitrogen required for domoic acid production.

The above experiments were carried out with nonaxenic cultures, and we can therefore not rule out the possible role of bacteria in the growth of *N. pungens* and/or in the production of domoic acid. Algal cultures in stationary phase typically release a large amount of dissolved organic matter (DOM). Since the *N. pungens* cultures were not axenic, it is possible that this DOM stimulated an associated bacterial flora, which then produced the observed domoic acid. For the moment, therefore, this problem remains unresolved. However, we have no evidence to support the hypothesis that bacteria, alone, produce domoic acid. For example, no particulate domoic acid was found in the retentate of a 0.2-μm pore size polycarbonate filter following filtration of a 3-μm filtrate from a culture medium in which *N. pungens* had grown (unpubl. data). Antibiotic treatments, both in our laboratory and in collaboration with two other laboratories (R. Selvin, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, pers. comm.; D. Douglas, Institute for Marine Biosciences, National Research Council of Canada, Halifax, N.S., pers. comm.), have resulted in

clones of *N. pungens* f. *multiseries* with no apparent bacteria in the growth medium. Experiments on the important question of domoic acid production by axenic cultures are in progress.

Interestingly, *N. pungens* cells remain viable for at least as long as 40 d in batch culture. The proportion of intact, chlorophyll-containing cells decreases during this period, usually not in excess of 2%·d^{-1} (unpubl. data), yet the viable cells that remain continue to produce domoic acid (Fig. 1), provided that nitrogen is still present in the medium after the culture reaches stationary phase. Furthermore, *N. pungens* blooms can persist for up to 3 mo in the field (Bates et al. 1989; Smith et al. 1990a). Is domoic acid accumulated as a nitrogen reserve that could be mobilized for the synthesis of essential amino acids, enabling *N. pungens* to remain viable for long periods of time during limitation by some other factor? Domoic acid is probably not as significant a nitrogen storage compound as is saxitoxin because it contains only 4.5% nitrogen on a molecular weight basis, compared with 33% nitrogen for saxitoxin produced by *Alexandrium* species (Boyer et al. 1987). Furthermore, the synthesis of domoic acid represented only 1.5%, on a molar basis, of the nitrogen taken up during the stationary phase in culture (Fig. 5). It is likely that the nitrogen taken up during the stationary phase is used to synthesize other nitrogen-containing compounds which, like domoic acid, are either stored or released into the medium. In another set of experiments (unpubl. data), no distinct photosynthetic advantage was seen for *N. pungens* f. *multiseries* during the stationary phase compared with the non-domoic-acid-producing form, *N. pungens* f. *pungens*. For now, the explicit ecological and metabolic role of domoic acid remains unknown.

The 1988 *N. pungens* bloom in eastern Prince Edward Island occurred concurrently with an increase in nitrate in the water column (Smith et al. 1990a). Although ammonium and urea are also important nitrogen sources for estuarine phytoplankton, it is evident from our laboratory results that the presence of nitrogen, in this case in the form of nitrate, was an essential factor for domoic acid production. Studies are in progress to determine the effects of ammonium-N on domoic acid production. Our finding that domoic acid was produced at similar rates at low and high irradiance levels is also consistent with an autumnal domoic-acid-producing bloom at a time when the ambient irradiance level was decreasing.

We conclude that at least three conditions are required for the production of domoic acid by our clone of *N. pungens* f. *multiseries* grown with nitrate: (1) cessation of cell division, (2) availability of nitrate or other suitable nitrogen sources during the stationary phase, and (3) the presence of light. Growth in culture medium f/2, which contains limiting silicate and excess nitrate, fulfils these requirements.

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Importance of Latitude and Organic Color on Phytoplankton Primary Productivity in Florida Lakes

John R. Beaver[1] and Thomas L. Crisman

Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611, USA

Beaver, J. R., and T. L. Crisman. 1991. Importance of latitude and organic color on phytoplankton primary productivity in Florida lakes. Can. J. Fish. Aquat. Sci. 48: 1145–1150.

A characterization of primary productivity patterns in subtropical Florida lakes along increasing gradients of both dissolved organic color and phytoplankton biomass is presented. Volumetric expression of gross primary productivity was more strongly correlated with chlorophyll *a* and nutrient concentrations than was areal expression. Primary production in clearwater (<75 Pt units) lakes was more predictable than colored (>75 Pt units) lakes. Areal production in Florida lakes was intermediate to the tropical and temperate regions, although volumetric productivity during the vegetative season (May–September) was not significantly different from temperate zone lakes for the same period. Predictive abilities of empirical equations describing primary productivity in Florida lakes are improved by distinguishing colored and clear lakes.

On présente la caractérisation des profils de productivité primaire dans des lacs de la Floride subtropicale en fonction de gradients croissants de la coloration due à la matière organique dissoute et de la biomasse phytoplanctonique. On a constaté que la productivité primaire brute exprimée en volume était en plus forte corrélation avec la concentration de chlorophylle *a* et la concentration de matières nutritives est plus grande que la productivité exprimée en superficie. La production primaire dans les lacs aux eaux limpides (<75 unités Pt) s'est révélée plus prévisible que dans les lacs aux eaux colorées (>75 unités Pt). La production par unité de superficie dans les lacs de la Floride se situait entre celle des lacs de région tropicale et des lacs de région tempérée, mais la productivité en unités de volume durant la saison végétative (mai–septembre) n'était pas significativement différente de celle des lacs de région tempérée durant la même période. La valeur prévisionnelle des équations empiriques de la productivité primaire des lacs de la Floride est meilleure lorsqu'on fait une distinction entre les lacs aux eaux colorées et ceux aux eaux limpides.

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It has long been recognized that phytoplankton productivity increases with increasing phytoplankton biomass in freshwater ecosystems. Brylinsky (1980) suggested that on a global basis, energy-related variables (i.e. solar isolation) are stronger determinants of primary production than are nutrient concentrations. Within geographical areas of narrow latitude, available phosphorus and nitrogen are better predictors of annual lake production (Smith 1979; Schindler 1978). Although Brylinsky (1980) reported that 57% of the variability in lacustrine gross primary production could be explained solely by latitude, the database he used from low-latitude lakes was rather limited.

The range of latitudes included by the above investigators spanned the equator to the arctic, but subtropical lakes were poorly represented. Based on the distribution of terrestrial flora and fauna, the Florida peninsula is climatically transitional between the warm temperate and subtropical zones (Dohwender and Harris 1975). We test the general validity of the latitude to gross primary production relationship by providing an assessment of lakes in subtropical Florida (>26°N–<31°N) whose total production should theoretically be intermediate with that reported for tropical and north temperate lakes (Lewis 1987).

[1]Author to whom correspondence should be addressed. Present address: Life Systems Inc., 24755 Highpoint Road, Cleveland, OH 44122, USA.

Unlike phosphorus and nitrogen, organic color has a variable effect upon phytoplankton populations. Dissolved humic material may sequester or release micronutrients (Guildford et al. 1987) and analagously may reduce or increase the toxicity of trace metals to algal cells (Wetzel 1983). Interactions between humic material and phosphorus and nitrogen forms also may regulate macronutrient availability (Guildford et al. 1987; Frankco and Heath 1983; Stewart and Wetzel 1982). Reduction in photic zone depth associated with high color levels can limit algal productivity (Arvola 1984). No systematic study, however, has related concentrations of organic color to primary productivity along a broad gradient of a trophy.

Thus our purpose is to (1) delineate patterns in primary productivity in Florida lakes relative to lakes in other climatic regions and (2) describe primary productivity to nutrient relationships for clear and colored lakes of Florida.

Methods

Data Sources

Records of simultaneous observations of gross primary productivity, chlorophyll *a*, organic color, total phosphorus (TP), and total nitrogen (TN) for Florida lakes were taken from the literature. Areal primary production values came from Cowell et al. (1975), Fontaine and Ewel (1981), McDiffett (1980), Milleson (1978), Maslin (1969), and Nordlie (1976),

TABLE 1. Limnological characteristics of Florida lakes used in volumetric primary productivity analyses (n = number of primary productivity measurements).

Lake	Chlorophyll *a* ($mg \cdot m^{-3}$)	TP ($mg \cdot m^{-3}$)	TN ($mg \cdot m^{-3}$)	Color (Pt units)	Secchi disk (cm)	Primary production ($mg \cdot cm^{-3} \cdot d^{-1}$)	n	Surface area (ha)	Mean depth (m)
Anderson Cue, 1967	5.2	16	410	6	310	54.5	27	59	2.0
Anderson Cue, 1969	3.2	19	510	3	371	14.9	6	59	2.0
Adaho	5.3	27	940	336	86	93.7	3	41	3.5
Alice	4.4	900	597	26	150	98.6	6	29	0.9
Altho	5.9	23	673	114	137	150.3	3	222	3.6
Annie	2.6	12	253	5	542	25.0	10	35	8.3
Apopka, 1969	60.4	380	5243	78	22	4 182.3	3	12 412	1.3
Apopka, 1977	45.8	193	3797	61	27	2 500.1	21	12 412	1.3
Arbuckle	25.6	14	872	175	94	709.7	4	1 154	2.4
Beauclair	93.2	208	3966	98	37	4 130.9	11	339	—
Brooklyn	1.9	11	387	4	430	9.9	3	261	5.7
Burnt	29.0	478	2317	352	61	3 299.3	3	58	1.5
Bevilles Pond	23.7	87	833	182	218	22.8	3	2	3.1
Bivens Arm	56.0	546	2098	42	61	3 299.3	6	58	1.5
Calf Pond	23.5	184	1870	404	71	165.1	3	11	1.6
Clay	9.5	4	720	24	262	409.6	4	110	5.3
Clear	26.4	392	1493	86	75	1 140.3	6	3	1.6
Clearwater	2.3	12	1377	21	244	5.9	3	5	1.5
Conway	4.4	—	—	20	—	284.6	13	559	—
Cooter Pond	22.6	165	1383	83	86	368.1	3	86	2.2
Cowpen	1.6	10	290	5	468	4.7	3	240	3.7
Dora, 1969	50.4	394	3692	97	38	3 891.9	6	2 237	3.0
Dora, 1977	76.0	147	3498	82	44	2 487.1	12	2 237	3.0
Elizabeth	8.0	36	937	237	60	80.9	3	27	1.5
Eustis, 1969	23.8	167	2443	47	76	1 174.8	3	3 159	4.1
Eustis, 1971	25.0	77	2507	38	46	1 315.7	9	2 159	4.1
Francis	18.1	27	572	82	26	193.8	10	218	2.9
Galilee	1.9	14	387	12	343	23.4	3	34	3.5
Geneva	1.4	2	443	10	312	32.8	6	660	4.1
Griffin, 1969	47.3	183	2823	36	67	2 755.1	3	3 533	2.4
Griffin, 1977	45.6	132	2955	67	57	2 741.9	11	3 533	2.4
Harris	14.5	37	1377	29	107	349.7	3	5 580	4.2
Hawthorne	56.8	79	1930	58	71	1 207.1	6	20	2.8
Hickory Pond	7.6	27	817	62	178	48.5	3	27	3.4
Istokpoga, 1975	15.7	17	987	157	67	314.1	4	8 382	2.3
Istokpoga, 1976	18.6	62	1248	110	—	1 115.3	24	8 382	2.3
Jeggord	7.0	59	550	193	81	82.9	3	64	3.0
Josephine	20.6	16	911	130	70	345.4	3	378	2.6
June-in-Winter	9.2	5	533	15	250	299.4	6	1 025	8.2
Kingsley	1.8	12	525	11	453	34.5	6	669	7.3
Kanapaha	42.7	212	2273	121	35	2 064.4	3	82	0.7
Lochloosa	23.3	58	1620	116	86	265.0	3	2 235	2.9
Long	1.4	12	157	10	414	3.5	3	104	3.4
Long Pond	12.6	64	1210	365	46	18.9	1	5	1.2
Little Orange	9.8	105	1143	166	94	270.0	3	314	2.8
Little Santa Fe	4.5	15	890	149	152	23.0	3	467	4.8
Magnolia	1.5	12	327	11	378	11.8	3	83	8.0
McCloud, 1968	7.2	8	370	6	560	71.5	11	6	2.0
McCloud, 1969	2.4	17	368	4	484	9.9	6	6	2.0
Meta	3.3	30	1120	25	224	27.9	3	2	1.6
Mize	33.9	113	2402	433	55	137.3	6	1	4.0
Moss Lee	8.0	36	953	98	142	306.8	3	52	3.6
Newnans, 1969	47.4	110	1840	189	53	1 271.7	6	3 006	1.5
Newnans, 1980	51.3	178	1983	104	58	10 940.3	18	3 006	1.5
Okeechobee	14.4	66	1802	26	—	1 322.5	82	132 893	4.0
Orange	15.6	63	1297	107	101	338.3	6	3 324	1.8
Placid, 1975	6.3	4	409	10	279	193.9	4	1 344	6.1
Placid, 1976	6.7	15	339	8	320	19.2	11	1 344	6.1
Palatka Pond	15.6	24	1240	93	49	109.8	3	4	0.8
Reedy	42.6	7	1424	17	80	737.5	3	1 398	3.3
Sand Hill	1.3	11	310	8	664	9.0	3	508	4.8
Santa Fe	5.6	21	643	59	234	117.7	6	1 674	5.5

TABLE 1. (*Concluded*)

Lake	Chlorophyll *a* ($mg \cdot m^{-3}$)	TP ($mg \cdot m^{-3}$)	TN ($mg \cdot m^{-3}$)	Color (Pt units)	Secchi disk (cm)	Primary production ($mg \cdot cm^{-3} \cdot d^{-1}$)	*n*	Surface area (ha)	Mean depth (m)
Santa Rosa	1.7	11	356	2	640	7.2	3	42	8.1
Still Ford	3.1	13	663	26	201	5.5	3	5	1.1
Suggs	3.4	36	870	281	92	84.2	6	47	2.5
Swan	1.6	11	295	6	320	39.6	6	227	4.8
Ten	12.6	64	1083	124	153	216.4	6	29	3.2
Thonotosassa	63.8	313	1391	50	82	624.7	5	331	2.5
Tuscawilla	11.32	350	1310	245	76	379.3	1	162	1.3
Twenty	92.8	410	2393	69	34	3 142.6	6	4	1.9
Twenty-five	3.5	28	783	36	152	29.9	3	6	1.0
Twenty-seven	30.1	325	857	92	140	15.6	3	5	3.8
Wall	5.1	25	833	151	137	84.1	3	31	2.1
Wauberg, 1969	37.3	169	1915	63	89	1 299.0	6	100	3.8
Wauberg, 1980	71.1	155	2109	17	46	18 392.2	13	100	3.8
Weir	6.4	19	1033	9	198	151.1	6	2 301	6.3
Winnott	2.6	23	747	23	338	16.6	3	85	5.2
Watermelon	9.0	52	987	74	96	226.1	3	213	1.5

and volumetric primary productivity values were taken from Brezonik et al. (1978), Crisman et al. (1986), McDiffett (1981), Marshall (1977), Shannon (1970), and Tuschall et al. (1979). Areal productivity was converted to a volumetric expression where sufficient morphometric detail was provided. Measurements obtained by both ^{14}C and oxygen light–dark bottle techniques were included. Productivity data were taken from a total of 68 lakes; however, in cases where multiple, nonoverlapping studies were made of the same lake, they were treated as separate entries (Table 1).

The lake set was divided into clear and colored subsets based on mean annual organic color, with 75 Pt units being the critical value for separation. The selection of 75 Pt units to classify our lakes was based on rapid light attenuation associated with color concentrations greater than this threshold (Brezonik 1978). Additional chlorophyll *a*, nutrient, and color data were taken from Beaver (1980), Bienert (1982), Brezonik et al. (1983), Cowell and Dawes (1984), Crisman et al. (1988), Garren et al. (1984), National Eutrophication Survey (1977), Russell (1982), Young (1979), and files of the Florida Water Resources Research Center.

Statistical Analyses

Prior to regression and correlation analyses, all variables were log $(n + 1)$ transformed. The SAS computer package (SAS Institute, Inc. 1985) was used for all statistical analyses.

Results and Discussion

Florida Lakes — Regression Models for Areal Production

Regression models relating annual areal productivity to chlorophyll *a*, TP, and TN had consistently poor predictive capacity (Table 2). The largest variation explained by the areal regressions was for chlorophyll *a* ($r^2 = 0.55$) and TN ($r^2 = 0.42$) in the clearwater subset. Regression coefficients for colored lakes were all <0.20 and were not significant. Chlorophyll *a* was the best overall predictor of areal annual production in Florida lakes. No additional variance in areal primary production was explained by multiple line regression using chlorophyll *a*, TN, TP, or color as independent variables.

TABLE 2. Regression models predicting annual areal gross production in Florida lakes (y = log annual production in g $C \cdot m^{-2} \cdot yr^{-1}$), x = log-transformed independent variable; chlorophyll *a* and TP in $mg \cdot m^{-3}$ and TN in $mg \cdot L^{-1}$).

Variable	Regression	r^2	*n*
Clear lakes			
Chlorophyll *a*	$y = 0.7x + 3.9$	0.55	15
TP	$y = 0.4x + 4.3^a$	0.21	15
TN	$y = 1.4x + 4.6$	0.42	15
Colored lakes			
Chlorophyll *a*	$y = 0.8x + 2.9^a$	0.20	6
TP	$y = 0.2x + 4.7^a$	0.03	6
TN	$y = -0.2x + 5.6^a$	0.01	6
All lakes			
Chlorophyll *a*	$y = 0.6x + 3.9$	0.38	21
TP	$y = 0.3x + 4.6^a$	0.10	21
TN	$y = 1.3x + 4.6$	0.28	21

[a]Not significant at $p < 0.05$.

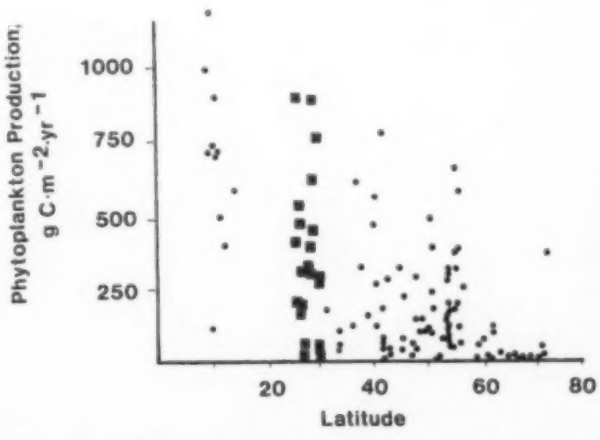

FIG. 1. Annual areal production in Florida lakes (squares) relative to those described by Brylinsky (1980) (circles).

TABLE 3. Regression models predicting annual volumetric gross production in Florida lakes (y = log annual production in g C·m^{-3}·yr^{-1}, x = log-transformed independent variable; chlorophyll *a* and TP in mg·m^{-3} and TN in mg·L^{-1}).

Variable	Regression	r^2	n
Clear lakes			
Chlorophyll *a*	$y = 1.7x + 0.1$	0.86	45
TP	$y = 1.0x + 0.3$	0.44	44
TN	$y = 4.7x + 0.8$	0.71	44
Colored lakes			
Chlorophyll *a*	$y = 1.4x + 0.5$	0.49	33
TP	$y = 0.7x + 1.6$	0.22	33
TN	$y = 3.4x + 1.7$	0.56	32
All lakes			
Chlorophyll *a*	$y = 1.6x + 0.3$	0.75	78
TP	$y = 0.9x + 0.6$	0.40	77
TN	$y = 4.1x + 1.1$	0.67	76

Annual production of 22 Florida lakes ranged from 22 to 901 g C·m^{-2}·yr^{-1} and averaged 358 g C·m^{-2}·yr^{-1}. These ranges in yearly production fit the established latitudinal trend relating increasing latitude to decreased amounts of absolute production (Brylinsky 1980). The most productive lakes in our data set were positioned intermediate between the maxima described for temperate and tropical regions, while the lower limit of production was independent of latitude (Fig. 1).

Florida Lakes — Regression Models for Annual Volumetric Production

Regression models for volumetric annual gross primary productivity against average chlorophyll *a*, TP, and TN concentrations in clear Florida lakes were highly significant ($p < 0.0001$), with more variation being explained than by the areal models (Table 3). The largest variance in annual productivity was explained by the chlorophyll *a* model ($r^2 = 0.86$). Models derived for TP ($r^2 = 0.44$) and TN ($r^2 = 0.71$) were less robust. Predictive models relating chlorophyll *a*, TP, and TN to primary productivity in colored Florida lakes were significant ($p < 0.05$), but less variance (chlorophyll *a* = 0.49, TP = 0.22, TN = 0.56) was explained. No significant difference existed between the regressions between chlorophyll *a* and productivities for clear or colored lakes, although the difference between slopes of the two lines did approach significance ($p < 0.075$) (Fig. 2). Comparison of slopes and intercepts for the TP equations demonstrated that the slope for colored lakes was significantly flatter ($p < 0.036$) than that of the clearwater subset, although the intercepts were not significantly different ($p < 0.083$). No difference was evident between the sets of equations using TN as an independent variable.

Regression models utilizing all data points were highly significant ($p < 0.0001$), with the majority of variation in gross primary productivity being explained by chlorophyll *a* concentrations ($r^2 = 0.75$). Stepwise linear regression using chlorophyll *a* and TN as independent variables improved the correlation coefficient to 0.81. TP and color did not contribute significantly to the model.

Mean annual color concentrations in Florida clearwater systems (<75 Pt units) were moderately ($r^2 = 0.53$) but significantly ($p < 0.0001$) related to estimates of mean annual volumetric productivity. No relationship was evident between color and productivity for the more highly colored (>75 Pt units) lake subset ($r^2 = 0.03$, $p < 0.4018$). The relationship between mean annual color and volumetric productivity was best described by a weak ($r^2 = 0.30$) but highly significant ($p < 0.0001$) hyperbolic function. An analysis of collinearity (SAS Institute, Inc. 1985) indicated that TP, TN, and color were strongly collinear with chlorophyll *a*, suggesting that the correlation between primary productivity and color was spurious or that color exerted negligible infuence on primary productivity.

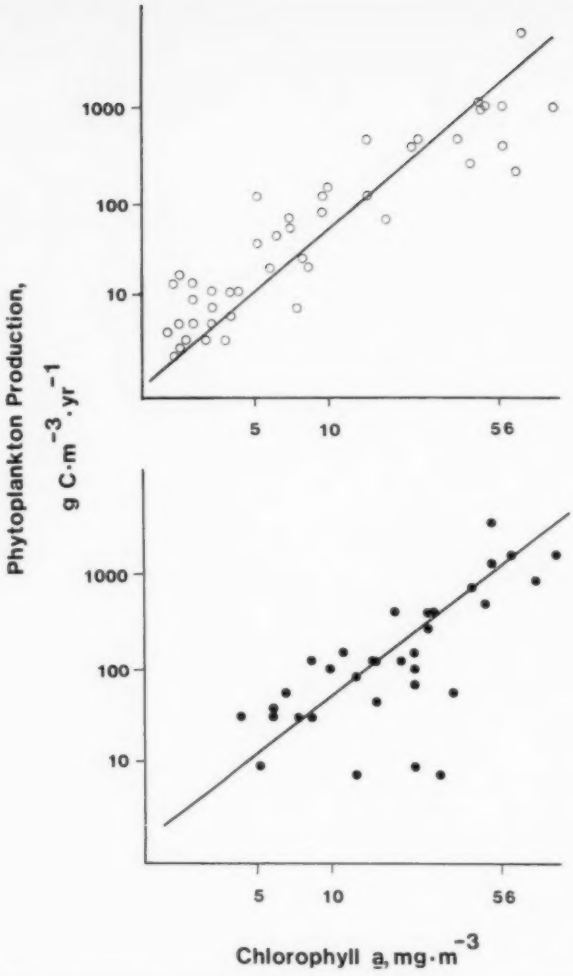

FIG. 2. Comparison of annual production in Florida clearwater (open circles) and colored (solid circles) lakes.

Although chlorophyll *a* is often used as a surrogate measure of phytoplankton biomass, our results caution against the overextension frequently made from chlorophyll *a* concentrations to "algal production" or "algal productivity," especially when dealing with systems rich in organic color. The presence of large amounts of organic color substantially reduces the predictive abilities of empirically derived equations (e.g. Granberg and Harjula 1982; Tolstoy 1988), particularly when it is assumed that the positive response of a phytoplankton community to increased TP and TN loadings will remain linear across the trophic spectrum without factoring in large changes in dissolved humic material. Recognition of the dystrophic lake

type (cf. Hansen 1962; Rodhe 1969) should markedly improve our understanding of nutrient–chlorophyll–productivity relationships.

Smith (1979) cautioned against expressing productivity on an areal basis rather than a volumetric basis. With increased eutrophication, corresponding shrinkage of the photic zone may limit integral photosynthetic production. Consequently, nutrient enrichment of the epilimnion may result in higher volumetric rates of algal production, but compression of the photic zone by algal turbidity mutes integral production values in some situations. Our results support Smith (1979), since prediction of areal primary production in Florida lakes was consistently poorer than volumetric production with clearwater lakes, irrespective of independent variable or color classification.

Comparison of Florida and Temperate Zone Lakes — Vegetative Season

Data compiled by Smith (1979) were used to determine if the phytoplankton communities in subtropical Florida lakes differed in their response to increased trophic state and primary production. Smith (1979) utilized vegetative season (May–September) means in TP, TN, chlorophyll *a*, and volumetric primary production collected from 49 predominantly clearwater (V.H. Smith, pers. comm.) temperate zone North American lakes to demonstrate a strong correlation between nutrients, phytoplankton biomass, and volumetric rates of photosynthesis.

Regression models for average daily production during the vegetative season (May–September) using mean chlorophyll *a* concentrations as independent variables for clear Florida lakes and temperate North American lakes were both highly significant ($p < 0.0001$) and had comparable regression coefficients ($r^2 = 0.81, 0.86$). Florida clearwater lakes generally exhibited similar production per unit chlorophyll *a* relative to temperate lakes. The slope of the regression for Florida lakes was statistically greater ($p < 0.0268$), but the intercept was lower ($p < 0.0740$). The 95% confidence intervals for both equations overlapped. The regression analysis was repeated excluding lakes with chlorophyll *a* concentrations >71 $mg \cdot m^{-3}$ (the maximum in Smith 1979) with no difference between paired equations. These results provide no evidence that subtropical phytoplankton assemblages differ relative to their production per unit biomass during the vegetative season when compared with other North American lakes at greater latitudes (Fig. 3).

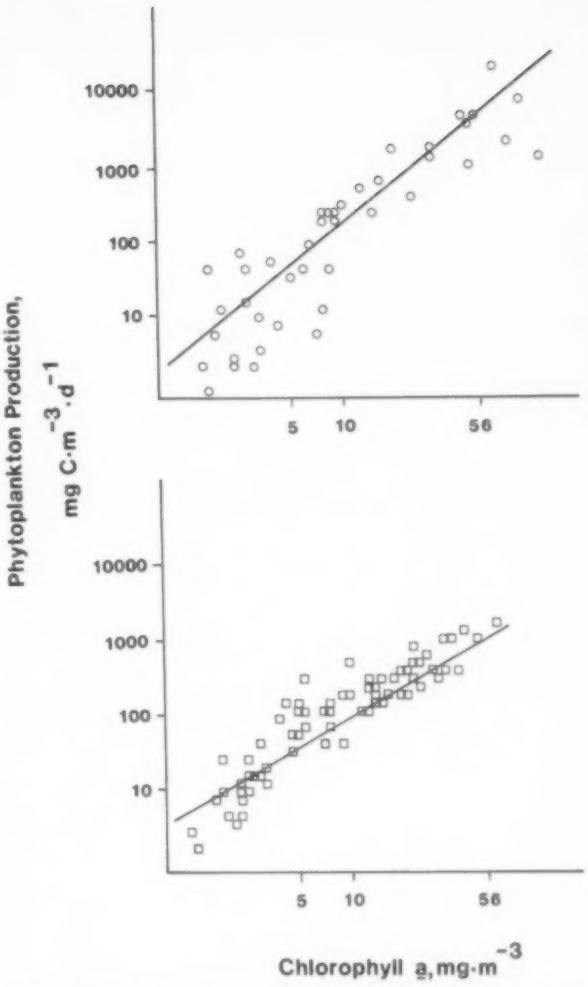

FIG. 3. Average daily primary production in Florida clearwater (open circles) and temperate zone lakes (open squares, taken from Smith 1979) during the vegetative season (May–September).

Florida lakes possess characteristics which fundamentally differentiate them from most temperate systems. The relative shallowness of Florida lakes combined with a moderate climatic regime dictate that stable thermal stratification usually only occurs in deeper (>5–6 m) lakes of the region (Beaver et al. 1981). Such deeper lakes are warm monomictic and circulate throughout the winter months, while shallower basins are more strongly affected by local meterological events, recirculate frequently, and are thus subjected to high internal nutrient loading via sediment resuspension (Pollman 1982). Higher solar insolation than the temperate zone and transitory stratification contribute to a year-round growing season with greatly reduced temporal variability in phytoplankton biomass and primary productivity compared with the temperate zone (Beaver and Crisman 1991).

Slopes for both the TN and TP to chlorophyll *a* regressions for Florida lakes are significantly flatter than those described for the temperate region (Baker et al. 1981), suggesting that subtropical systems can assimilate greater amounts of nutrients compared with temperate lakes before a given response is observed in phytoplankton biomass. Recently, Agusti et al. (1990) suggested that nonnutrient constraints such as self-shading may partially regulate phytoplankton biomass in eutrophic (chlorophyll $a > 10$ $mg \cdot m^{-3}$) Florida lakes. This is in accord with the general poor predictive ability of TP and TN in describing primary production for Florida lakes.

Areal annual primary production in Florida lakes was intermediate to the established latitudinal pattern which strongly related solar energy variables to total phytoplankton production (Brylinsky 1980). At a given level of trophy, decreasing latitude should result in increased absolute system production (Lewis 1987). Patterns in growing season production in temperate North American and subtropical lakes do not contradict this trend — spring and summer meterological conditions in North America display no marked latitudinal discontinuity as in other seasons. While production in subtropical systems is somewhat depressed during winter, many temperate systems are covered with snow. Thus, if nonvegetative season estimates

of production are included in determinations of annual production, it is likely that subtropical systems would realize greater yearly production.

Variability in annual primary production relative to trophic status encountered in the colored lake subset supports the contention that increasing color concentrations do not have a clear positive or negative influence on phytoplankton biomass and metabolism (Canfield et al. 1984). It is likely that mechanisms such as regulation of micro- and macronutrient availability operate in colored lakes simultaneously and temporally to structure the biomass and metabolic characteristics of phytoplankton populations. Our results provide no evidence that organic color exerts a systematic effect on phytoplankton production across a trophic gradient.

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Indexing Deformities in Ligulae and Antennae of *Procladius* Larvae (Diptera: Chironomidae): Application to Contaminant-Stressed Environments[1]

W. F. Warwick

National Hydrology Research Institute, 11 Innovation Boulevard, Saskatoon, Sask. S7N 3H5, Canada

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The severity of deformities in the ligulae and antennae of *Procladius* is indexed and both indices are expanded to include population and community indices and a general index of Chironomid Community Response. These biological indices are compared with Indices of Contamination derived from chemical data from the St. Lawrence River, Port Hope Harbour, Southern Indian Lake – Notigi Reservoir, Tobin Lake, and Last Mountain Lake. Biological and chemical indices both identified Lac St. Louis Site 4 on the St. Lawrence River as the most highly contaminated site and the Wupaw Bay site in the Southern Indian Lake – Notigi Reservoir as the least contaminated of the 23 sites studied. The increase in frequency and severity of deformities in spring and summer data from the Rat Lake basin suggests that larvae are sensitive to increased mercury availability resulting from the greater activity of mercury-methylating bacteria in warmer summer waters.

On a établi deux indices d'après la gravité des déformations de la ligule et des antennes chez *Procladius*, indices qu'on élargit pour y intégrer des indices de population et de communauté ainsi qu'un indice générale de la réaction de la communauté de chironomides. On compare ces indices biologiques à des indices de contamination déterminés d'après les caractéristiques chimiques des eaux du Saint-Laurent, de Port Hope, du lac Southern Indian – réservoir Notigi, du lac Tobin et du lac Last Mountain. D'après les indices biologiques et chimiques, sur les 23 stations étudiées, c'est à la station 4 du lac Saint-Louis dans le Saint-Laurent que les eaux sont le plus contaminées, tandis qu'à la station de la baie Wupaw dans le lac Southern Indian – réservoir Notigi, elles sont le moins touchées. L'augmentation de la fréquence et de la gravité des déformations au printemps et en été dans le bassin du lac Rat indiquent que les larves sont sensibles à l'élévation de la concentration de mercure qui se produit en été lorsque, les eaux étant plus chaudes, les bactéries productrices de mercure sont plus actives.

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Biological approaches to the assessment of water quality and the health of freshwater ecosystems offer a number of advantages over chemical and/or bioassay approaches alone. Biological methods represent a systems approach that focuses on the resource and its response to different environmental impacts. In contrast, chemical/bioassay methods represent a regulatory approach that focuses on specific pollutants with specific rules for their discharge into the environment (Ohio Environmental Protection Agency 1987). Biological communities are good indicators of aquatic conditions because they are intimately bound to their environment and are directly subject to the sum total of all chemical, physical, and biological processes influencing that environment over the length of their life cycles. On the other hand, chemical measurements, and to a lesser degree, bioassay measurements, are biased toward short-term conditions that exist only at the time of sample collection (Warwick 1990a).

The Chironomidae possess a number of advantages as indicators of aquatic conditions (Warwick 1990a) and have been used in the lake classification system to categorize lakes according to trophic state (Brundin 1949; Saether 1975; Warwick 1980). As an indicator community, chironomids obviate a number of the limitations of single indicator species because they represent all functional groups within their environment (Dayton 1984). Pinder (1986) regarded the interpretation of morphological responses to contaminants as a direct extension of the lake classification system.

Several years ago, I proposed using morphological deformities to detect and assess the effects of long-term, chronic toxicity and began a systematic evaluation of these responses in chironomid larvae (Warwick 1985). An indexing to assess the severity of deformities in the antennae of *Chironomus* was developed in Warwick (1985) and deformities in the other structures of the head capsule of *Chironomus* were categorized by Warwick and Tisdale (1988); in the latter we also described deformities in some head capsule structures of *Cryptochironomus* and *Procladius* larvae. Deformities in the ligula, paraligula, mandible, dorsomentum, and hypopharyngeal pecten of *Procladius* were categorized in Warwick (1989). A scheme adapted from Warwick (1985) for indexing deformed antennae in *Procladius* was also included.

I have adopted this approach because I believe that severity of response yields more information about response to contaminant stress than the usual method of expressing biological characteristics in terms of percentage or frequency of occurrence (e.g. see National Research Council of Canada 1985). For example, the normal mentum of *Chironomus* has a complement

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of 15 teeth. In deformed larvae, the impact of the presence or absence of a single tooth cannot compare in either form or function with menta having in excess of 50 teeth, and yet, in terms of frequency, both would be equivalent.

In this paper, my objectives include (1) presenting an extended indexing scheme for evaluating deformities in the ligulae of *Procladius*, (2) comparing its efficacy at a number of sites experiencing differing degrees of contaminant stress, (3) applying and assessing the Index of Severity of Antennal Deformation (ISAD) to *Procladius*, and (4) examining some of the factors which may contribute to the impact of contaminants reflected in these deformities, and ultimately on the entire chironomid community.

Materials and Methods

The larvae used in this study came from a number of sites in eastern and western Canada. Locations and contaminant status are given in Warwick (1989, Table 1) and most sites are discussed in some detail in Warwick (1989, 1990b). Two additional sites in Tobin Lake (Sites V and VI) have been included here from Warwick (1985).

St. Lawrence River

The St. Lawrence River material comes from the river–lake area immediately opposite Montreal, Quebec (Warwick 1989). Three sites are located within the confines of the St. Lawrence Seaway off Laprairie, Quebec, seven are in Lac St. Louis, and two are sites in man-made ship canals. The Laprairie basin sites are separated from the main river by the dikes of the St. Lawrence Seaway system and thus are poorly flushed. The three sites monitor an increasingly severe trophic gradient caused by the introduction of untreated sewage from Laprairie. Conditions deteriorate progressively from Site 3 near the Champlain Bridge through Site 7a to Site 13a immediately offshore from the sewage treatment facility at Laprairie.

The Lac St. Louis sites include Site 2 located near the confluence of the St. Lawrence and Ottawa rivers, Sites 3, 4, 5, and 6 located in a small bay immediately offshore from the industrial complex around Beauharnois, Quebec, Site 7 located eastward off the mouth of the St. Louis River, and Site 8 located eastward again in a bay protected from the main flow of the St. Lawrence River by the island and reef complex of Îles de la Paix (Warwick 1989).

The man-made sites include Site 1 located in the ship-mooring basin above the St. Catherine locks, the locks linking Lac St. Louis and the Laprairie basin, and Site 9 located at the upper end of the old Lachine Canal. Site 1 is dredged, comparatively deep, and most likely exposed to contaminants from ships in transit through the canal. Site 9 is located in the protected basin at the entrance to the Lachine Canal which now serves as an anchorage for a local yacht club (Warwick 1989).

Lac St. Louis receives contaminants from the highly industrialized, international section of the St. Lawrence River and from the Ottawa River. Local point sources along the southern side of the lake also contribute heavy metals (Table 1), including mercury from a chloralkali plant on the St. Louis River (Sérodes and Talbot 1978) and organic contaminants (Table 2) from the industrial complex around Beauharnois (Champoux and Sloterdijk 1988). Elevated heavy metal and organic contaminant concentrations have been reported from the sediments (Sérodes 1978; Jarry et al. 1985), water (Germain and Janson 1984), aquatic plants (Béland and Demers 1977), benthos (Levasseur 1977), and fish (Sloterdijk 1977; Guay et al. 1986). The Laprairie basin is contaminated by heavy metals (Table 1), and probably organic contaminants as well, but also suffers the additional problem of untreated domestic wastes introduced from the Laprairie sewage treatment facility.

Southern Indian Lake – Notigi Reservoir

The Southern Indian Lake – Notigi Reservoir material includes larvae from four sites exposed to varying degrees of water level change and erosion of sediments as a result of beachline reestablishment following reservoir filling (Warwick 1986). Prior to flooding, Wupaw Bay was shallow and prone to wind stress. Although water level increases (3.1 m) have resulted in some minor bank erosion, increased water depths appear to have helped stabilize the bottom environment of silty-clay by reducing exposure to wind action. In contrast, similar water level increases (3.1 m) have severely impacted South Bay where the broad fetch of wind has caused severe shoreline erosion. Large amounts of mineral soils and organic debris from the forest floor have eroded from exposed shorelines into the bay. The sediments of South Bay are classified as clayey-silts, but this is misleading. Shoreline materials erode into the bay as "clay balls" ranging in size from 2 cm in diameter to sand and silt sizes (Hecky and McCullough 1984), and these aggregates tend to bias particle-size measurements toward the coarser end of the scale. With proper diagenesis, these sediments probably would more properly be classified as silty-clay sediments.

The Central Mynarski Lake basin has been exposed to greater changes in water depth (6.9 m), but only minor bank erosion appears to have occurred. The sediments at this site are highly organic and finely divided to the extent that they are classified as clay-sized sediments (Warwick 1986). The Rat Lake basin has experienced greater increases in water depth (15.2 m), but here the particle-size distribution shows evidence of greater erosion stress. The characteristic tail (~16 %) of very fine material (greater than 11.50 phi) in these sediments probably represents either newly eroded bank material, which because of the site's greater depth has achieved better sorting than the South Bay material, or mineral sediments borne in by the nearby Suwannee River (Warwick 1986).

Concern for the Southern Indian Lake – Notigi Reservoir environment developed when mercury levels in fish rose above commerically acceptable levels (Bodaly and Hecky 1979; Bodaly et al. 1984b). The mercury apparently comes from natural sources, and the degree to which concentrations have increased correlates with the extent of areal flooding in the different basins of the reservoir (Bodaly et al. 1984a). The highest concentrations of mercury in the reservoir were measured (Table 3) in the sediments of Central Mynarski Lake and closely approximate the Ontario Ministry of the Environment (MOE) maximum-level criterion of 0.3 $\mu g\ Hg \cdot g^{-1}$ in sediments. Although mercury has been identified as the problem of concern in Southern Indian Lake – Notigi Reservoir, concentrations for both copper and cadmium also exceed MOE criteria for metals in sediments.

Last Mountain Lake

The Last Mountain Lake material includes specimens collected off Saskatchewan Beach (Site I) and Rowan's Ravine (Site II) on the east–west and north–south arms of the lake, respectively (Warwick 1985, 1989). The Qu'Appelle River,

TABLE 1. Trace metal concentrations ($\mu g \cdot g^{-1}$) in the Lac St. Louis (from Champoux and Sloterdijk 1988) and Laprairie (from Sérodes 1978) basins. Values exceeding MOE criteria are underlined. (— = no data)

	Lac St. Louis (1985)				Laprairie (1976)			
	L-12 (near Site 2)	L-21 (ca. Sites 3–6)	L-2 (Site 7)	L-6 (Site 8)	Site 3	Site 7	Site 13	MOE criteria
Lead	65	101	40	27	100	45	38	50
Mercury	0.45	2.25	1.38	2.20	0.09	0.75	2.77	0.30
Chromium	—	—	92	60	53.0	75.5	80.5	25
Zinc	370	686	155	142	127	300	342	100
Nickel	55	50	45	32	32.8	49.6	48.4	25
Copper	68	64	46	40	30.9	55.9	53.5	25
Beryllium	—	—	—	—	1.46	1.67	1.52	—
Cobalt	—	—	16	3	—	—	—	50
Vanadium	—	—	104	53	—	—	—	—
Arsenic	6.39	12.90	5.66	5.33	—	—	—	8
Cadmium	1.70	14.00	1.40	1.00	—	—	—	0.10
Selenium	1.23	1.10	0.77	1.14	—	—	—	—

TABLE 2. Organic contaminant concentrations ($\mu g \cdot g^{-1}$) for comparable sites in Lac St. Louis, 1985 (from Champoux and Sloterdijk 1988). (— = no data)

	L-12 (near Site 2)	L-21 (ca. Sites 3–6)	L-2 (Site 7)	L-6 (Site 8)
Hexachlorobenzene	<0.004	0.015	0.008	<0.004
Anthracene	0.040	—	0.020	0.001
Fluoranthene	0.030	—	<0.001	0.010
Pyrene	<0.001	—	0.250	0.020
Chrysene	0.080	—	<0.001	0.040
Naphtalene	0.050	—	0.040	<0.010
Acenaphthylene	<0.020	—	0.220	<0.020
Phenanthrene	0.020	—	0.110	<0.001
Benzo[*a*]anthracene	0.007	—	0.550	0.009
Benzo[*b*]fluoranthene	0.070	—	0.280	0.030
Benzo[*k*]fluoranthene	0.010	—	0.080	0.010
Benzo[*a*]pyrene	0.030	—	0.240	0.020
Dibenzo[*ah*]anthracene	0.020	—	0.800	0.090
Benzo[*ghi*]perylene	0.040	—	0.650	0.040
Indeno[123*cd*]pyrene	0.050	—	0.480	0.050
Total PCB's	<0.090	0.110	<0.090	<0.090
Total PAH's	0.447	—	3.720	0.320

TABLE 3. Trace metal concentrations ($\mu g \cdot g^{-1}$) for sites in Southern Indian Lake – Notigi Reservoir, Tobin Lake, and Port Hope Harbour, Lake Ontario. Values exceeding MOE criteria are underlined. (— = no data)

	Southern Indian Lake – Notigi Reservoir					Tobin Lake		
	Rat Lake		Mynarski Lake	South Bay	Wupaw Bay			Port Hope
	Spring	Summer	(summer)	(summer)	(summer)	Site 2	Site 17	Harbour
Lead	—	—	—	—	—	15.0	25.0	483.0
Mercury	0.104	0.113	0.250	0.055	0.108	0.084	0.046	—
Chromium	—	—	—	—	—	89.0	84.0	50.0
Zinc	100.0	70.8	66.0	72.5	59.6	77.0	91.0	205.7
Nickel	—	—	—	—	—	11.0	36.0	58.0
Copper	11.6	19.4	43.6	22.3	11.8	12.0	30.0	122.0
Beryllium	—	—	—	—	—	—	—	—
Cobalt	14.6	46.5	18.0	45.8	27.4	11.0	9.5	57.0
Vanadium	—	—	—	—	—	—	—	33.0
Arsenic	—	—	—	—	—	7.7	6.9	—
Cadmium	0.1	1.9	2.5	1.2	1.5	0.2	0.4	1.2
Selenium	—	—	—	—	—	—	0.5	0.7

TABLE 4. Levels of radionuclides in surficial sediments of the basin and west slip of Port Hope Harbour, Lake Ontario (from Hart et al. 1986).

Parameter	Unit	Mean concentration (n = 13)	Concentration range
U	$\mu g \cdot g^{-1}$	92.5	3.5–1280
Th-230	$Bq \cdot g^{-1}$	6.78	0.063–76.6
Ra-226	$Bq \cdot g^{-1}$	1.56	0.031–297
Pb-210	$Bq \cdot g^{-1}$	6.54	0.60–682
Po-210	$Bq \cdot g^{-1}$	5.79	0.30–739
Th-232	$Bq \cdot g^{-1}$	0.20	0.015–5.2
Th-228	$Bq \cdot g^{-1}$	0.10	0.014–2.2

which drains Last Mountain Lake, is contaminated by wastes from the cities of Regina and Moose Jaw, Saskatchewan. In the spring, water from the river is shunted back up into the lake to alleviate downstream flooding in the Qu'Appelle Valley (Warwick 1985). Contaminants from this source probably are confined to the lower reaches of the lake (Site I) and do not impact Site II to any great degree. Both sites probably do receive pesticide and herbicide residues from the surrounding agricultural area, however (Warwick 1985). No chemical data are currently available for this lake.

Tobin Lake

The Tobin Lake material includes specimens from four sites. Site 2 is located just below the inflow of the Saskatchewan River into the Tobin Lake reservoir and is directly exposed to the full impact of the contaminant and sediment burden from the greater prairie region whereas Site 17 is located in a flooded backwater area and probably receives contaminants only indirectly (Warwick 1985, 1989; Warwick and Tisdale 1988). Mineral sediment accumulation from flooded agricultural areas flanking the old Petaigan River Valley may be considerable at Site 17 because the shallow areas are heavily wind stressed and subject to drawdown exposure. The two Tobin Lake sites added in this report are Sites V and VI from Warwick (1985). These sites lie in the old submerged Saskatchewan River channel towards the lower end of the reservoir. The sediments of both sites are highly organic, but the sediments at Site VI were particularly noteworthy for the large quantities of wood-pulp fibriles, probably from pulp and paper mills upstream.

Four metals, cadmium, chromium, copper, and nickel, exceed MOE criteria for metal concentrations in sediments at Site 17 whereas only two, cadmium and chromium, exceed these criteria at Site 2 (Table 3). Although one might expect higher metal concentrations at the latter site, as much as 8 m of silt has been deposited in this area since impoundment in 1964 (Crippen Acres Limited 1979). Rapid mineral sediment accumulations can depress element or contaminant concentrations, and the very rapid rates of accumulation in this delta area probably affect the concentration of these elements, as well as their impact, through dilution and reduced availability (Warwick 1980).

Port Hope Harbour

The Port Hope Harbour material includes specimens from three sites located in the inner harbour (Sites B, C, and E; Cook and Veal 1968). The basin is heavily contaminated by radionuclide wastes, cooling water, and trace metals from the nearby uranium refinery and the yacht club and adjoining townsite (Hart et al. 1986; Warwick et al. 1987). Seven metals, lead, chromium, zinc, nickel, copper, cobalt, and cadmium, exceeded MOE criteria for metals in sediments (Table 3). No criteria are available for vanadium or selenium. Radionuclide concentrations in the sediments are presented in Table 4.

Bay of Quinte

The Bay of Quinte material includes contemporary and subfossil specimens recovered from a sediment core collected in 1972 near Glenora, Ontario (Warwick 1980).

Analytical Procedures

Chemical data from published and unpublished sources were used to calculate an Index of Contamination (I_c) for each site following Champoux and Sloterdijk (1988) such that

$$(1) \quad I_c = \frac{1}{k} \sum_{i=1}^{k} \frac{(\text{teneur})_i}{(\text{critère})_i}$$

where "teneur" is the concentration of contaminant in the surficial sediment, "critère" is the not-to-exceed concentration for that contaminant established by MOE, and k is the number of contaminants used in the calculation. A value less than 1 signifies that, on average, the concentration of contaminants is less than the MOE criteria whereas a value greater than 1 indicates contamination exceeding MOE criteria.

Benthic samples were collected with a tall 232-cm^2 Ekman dredge, sieved through a 200-μm-mesh sieve net of Nitex nylon, and preserved in 70% ethanol. The preserved samples were stained with Rose Bengal, sorted under a steromicroscope, and separated into individual taxa. Chironomid larvae were mounted in Canada balsam using the procedures outlined in Warwick (1985, 1988). These methods have been developed especially for examination of fine morphological detail using high-power oil immersion microscopy. All drawings were done at 100× magnification using a Wild M-20 microscope equipped with a camera lucida apparatus.

Subfossil chironomid remains were recovered from the sediments of the Glenora-B core using the techniques detailed in Warwick (1980). In brief, a measured amount of sediment was digested in warm 8% potassium hydroxide, rinsed through a fine-mesh sieve, and the residue rewashed in a solution of potassium hydroxide and trisodium orthophosphate. The washed residue was then dehydrated through a series of ethanol baths and stored in anhydrous ethanol for sorting under stereomicroscope. Sorted specimens were mounted in euparal following procedures similar to those outlined above.

Indexing the Severity of Ligula Deformation

The normal ligula of *Procladius* (Fig. 1.1) has five dark brown to black teeth which are apically concave and mounted flexibly on the prementohypopharyngeal complex. It is flanked on either side at the base by leaf-shaped paraligulae. The ligula is analogous, in part, to the mentum in other chironomid subfamilies (Saether 1980). It is laminated, and uneven wear or breakage of the upper and lower edges make broken or worn teeth easy to detect.

Assessment of the severity of deformation is based on an arbitrarily established geometric ranking scheme which intuitively approaches the effect on function of increasingly severe

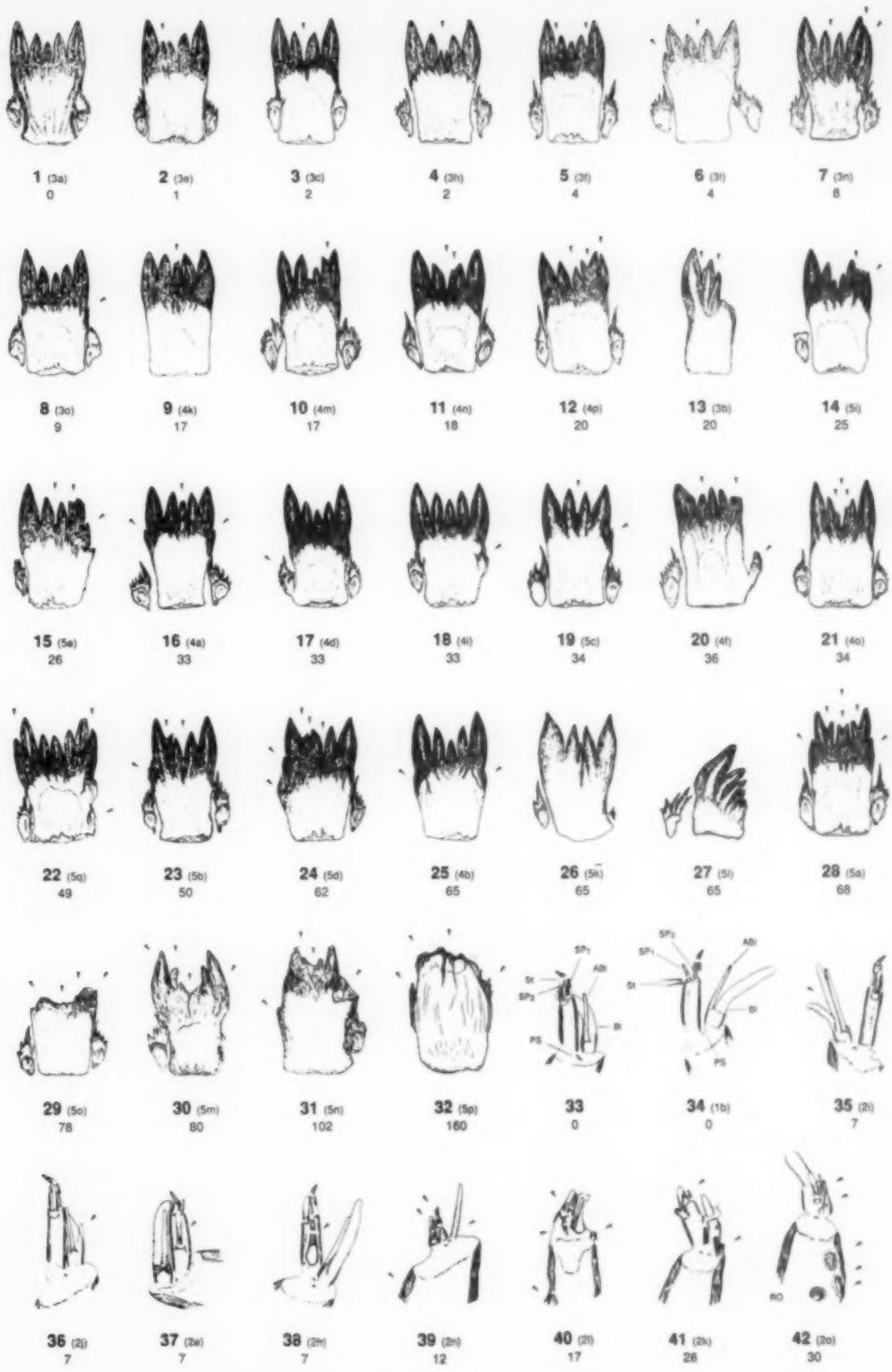

FIG. 1. Deformities in ligulae and antennae of *Procladius* larvae: 1.1, normal ligula; 1.2–1.32, deformed ligulae; 1.33–1.34, normal antennae; 1.35–1.42), deformed antennae. Abbreviations: Bl, blade; ABL, accessory blade; PS, peg sensilla; St, style; SP_1, SP_2, sensory pegs; RO, ring organ. Numerical identifications: bold = figure number; parenthetical = figure number from Warwick (1989); below = IMR evaluation.

TABLE 5. Basic classification categories and values for indexing individual types of deformities in the ligulae of *Procladius* larvae.

Step	Feature	Basic index number
1	Number of teeth	
	5 teeth	1
	4 or 6 teeth	2
	3 or 7 teeth	4
	2 or 8 teeth	8
	1 or 9 teeth	16
	0 or 10 teeth	32
	>10 teeth	?
2	Modification of larger, outer teeth; each	2
3	Overlapping of teeth: each	4
4	Presence of accessory teeth: each	8
5	Presence of forked teeth: each	16
6	Fusing of ligula and paraligula: each	32
7	Massive disorganization	64
8	Asymmetrical	64

deformation. Although difficult to test, one might expect that a larva with a grotesquely deformed ligula would be more seriously handicapped than a larvae whose ligula lacks only a single tooth. The ranking scheme presented here tries to take this into account. Compared with a simple linear scale, a geometric scale also provides better separation between categories of deformation.

The ranking scheme is formatted (Table 5) according to the framework presented in Warwick (1989). Eight basic categories are used to assess the severity of ligula deformation as follows.

Step 1. Number of Teeth

This category is based on those teeth in a deformed ligula following the classical apically concave pattern. Within this pattern, deformed ligula may have numbers of teeth less than, greater than, or equal to the normal complement of five teeth. The ranking value for each deviation from the normal number of teeth begins with a value of 1 for a ligula with five teeth, but one of which is abnormal (Fig. 1.2), and doubles thereafter for each tooth lost or added to the ligula. Thus, ligula with four (Fig. 1.3) or six (Fig. 1.4) teeth are assigned a value of 2 whereas those with two extra, or a total of seven teeth (Fig. 1.5), are assigned a value of 4. In the extreme case where no recognizable teeth are present (Fig. 1.32), the value assigned under the category "number of teeth" would be 32. At the other end of the scale, that is, in those ligula with additional teeth, the upper limit is not constrained.

Step 2. Modification to the Larger, Outer Teeth

This category involves the reduction in size of the larger, outer teeth of the ligula. Because the reduction in size places these teeth outside the classical apically concave pattern, and because extreme examples sometimes can be difficult to separate from accessory teeth (see below), modified outer teeth are treated separately. In Fig. 1.6, only four teeth follow the classical pattern whereas one outer tooth is modified in size and length. The value assigned a reduced outer tooth is 2.

Step 3. Overlapping of Teeth

This category is based simply on the number of overlapping teeth, each of which is assigned a value of 4. In Fig. 1.7, the ligula displays a single overlap between the two left-hand outer teeth, the outermost of which is reduced, and a complement of four teeth following the classical concave pattern for *Procladius*.

Step 4. Presence of Accessory Teeth

Accessory teeth are small teeth mounted high on the inner waisted curve of the ligula (Fig. 1.8). These teeth can sometimes be difficult to differentiate from extreme examples of modified outer teeth or fusing between the ligual and paraligula. Colouration usually provides the key for distinguishing between the types with, as in this example, colouration of accessory teeth being continuous with that of the main teeth. Each accessory tooth is assigned a value of 8. The ligula in Fig. 1.8 has one accessory tooth and therefore is assessed one point for being deformed plus eight for a total of 9 points.

Step 5. Presence of Forked Teeth

Forking can occur in any one (Fig. 1.9–1.12) or more (Fig. 1.28) of the teeth on the ligula. Each occurrence is assigned a value of 16. Forked teeth can occur singly (Fig. 1.9, 1.10) or in combination (Fig. 1.11–1.16) with other types of deformities including the extreme example in Fig. 1.28 where the scores for the three forked teeth account for 48 of the total of 68 index points assessed this ligula.

Step 6. Fusing between the Ligula and Paraligula

Fusing between the ligula and paraligula can take several forms ranging from pedestal-mounted paraligula (Fig. 1.17, 1.20), to toothed paraligula fused to the ligula which replace (Fig. 1.18) or supplement (Fig. 1.19) the normal complement of paraligula, to simple or untoothed paraligulae fused directly to the side of the ligula (Fig. 1.25). The latter example (Fig. 1.25) illustrates a case where differentiation between accessory teeth and fused paraligula can become problematic. In this case, however, there is a distinct colour separation between the paraligula and the main body of the ligula which makes differentiation possible. The ligula in Fig. 1.24 is an example of the two types of deformity occurring side by side. Presently, each instance of fusing is assigned a value of 32. As more information becomes available, it may be possible to further distinguish between the types of fusing.

Step 7. Massive Disorganization

The massively deformed ligulae shown in Fig. 1 were all found in sites experiencing heavy contamination; two (Fig. 1.29, 1.32) came from sites immediately offshore from the industrial complex at Beauharnois and two (Fig. 1.30, 1.31) came from Tobin Lake Site 2 where exposure to the contaminant burden of the Saskatchewan River is most direct. Massively deformed ligulae are assigned a base value of 64.

Step 8. Asymmetry

Ligulae displaying extensive asymmetry are also shown in Fig. 1. Those in Fig. 1.26 and 1.27 both occurred in very early instar larvae, which suggests that this type of deformity may result from interference in the transition process between early

TABLE 6. Examples of estimating the Index of Morphological Response (IMR) for ligulae of *Procladius*.

	Number of teeth											Modifications of outer teeth		Overlapping teeth			Accessory teeth			Forked teeth			Fusing of ligula and paraligula		Massive or asymmetrical teeth			
Fig.	0	1	2	3	4	5	6	7	8	9	>9	Left	Right	1	2	>2	1	2	>2	1	2	>2	Left	Right	Mass.	Asym.	Other	IMR
1.1																												0
1.2						1																						1
1.3					2																							2
1.4							2																					2
1.5								4																				4
1.6					2								2															4
1.7					2							2		4														8
1.8						1											8											9
1.9						1														16								17
1.10						1														16								17
1.11					2															16								18
1.12					2							2								16								20
1.13				4																16								20
1.14						1											8			16								25
1.15					2												8			16								26
1.16						1											8	8		16								33
1.17						1																		32				33
1.18						1																	32					33
1.19					2																		32					34
1.20					2							2											32					36
1.21					2																32							34
1.22						1												16					32					49
1.23					2															16				32				50
1.24					2									4			8			16				32				62
1.25						1																	32	32				65
1.26						1																				64		65
1.27						1																				64		65
1.28				4														16				48						68
1.29			8									2		4											64			78
1.30										16															64			80
1.31					2									4										32	64			102
1.32	32																						32	32	64			160

instar stages. This type of deformity is similarly assigned a base value of 64.

Index of Morphological Response

An Index of Morphological Response (IMR) is calculated for each deformed ligula according to the eight basic steps outlined above (Table 6; compare Fig. 1.2–1.32). IMR represents the cumulative total of the values assessed each category of deformity, singly or in combination, as shown in the following examples of severely deformed ligulae. The ligula in Fig. 1.31 has four teeth (2), one of which is overlapped (4), one fused paraligula (32), and is massively deformed (64) for an IMR value of 102. The second example in Fig. 1.32 has no readily distinguishable teeth (32), both paraligula are fused (64), and is massively deformed (64) for an IMR value of 160.

Index of Severity of Ligula Deformation (ISLD) at Population and Community Levels

ISLD is interpreted as a measure of the morphological response to stress exhibited by the ligulae in a *Procladius* population exposed to toxicants in the aquatic environment and is defined simply as

$$(2)\quad \mathrm{ISLD} = \frac{\sum \mathrm{IMR}}{n}$$

where ΣIMR is the sum of individual IMR values for a *Procladius* population divided by n, the number of *Procladius* larvae examined in that sample or site.

A new calculation is proposed to broaden the index to include the total chironomid community:

$$(3)\quad \mathrm{ISLD}_{\mathrm{Total}} = \frac{\sum \mathrm{IMR}}{N}$$

where ΣIMR is now divided by N, the total number of chironomid larvae examined in that sample or site.

Indexing the severity of antennal deformation

The antenna of normal *Procladius* has been described in detail in Warwick and Tisdale (1988, fig. 13) and Warwick (1989, fig. 1). In brief, it has four segments, the basal segment of which is considerably longer than the total length of the three remaining segments. The basal segment is equipped with a ring organ distally, but apparently no basiconic sensillum. The blade/accessory blade complex is located on the articulating membrane between the first (basal) and second antennal segments (Fig. 1.33, 1.34). Two tiny reniform structures also located on this membrane have tentatively been identified as peg sensilla. Three structures are located at the apex of the second segment, the larger of which is a heavy-walled structure similar to the style in *Chironomus*. The two smaller tubular structures (referred to as sensory pegs SP_1 and SP_2 in Roback 1980) are mounted on individual pedestals rather than directly on the articulating membrane linking Segments II and III.

Warwick's (1985) ranking scheme which uses six basic categories for indexing antennal deformities was revised as shown

TABLE 7. Basic classification categories and values for indexing individual types of deformities in the antennae of *Procladius* larvae (modified from Warwick 1985).

Step	Feature	Basic index number
1	Basic classification categories	
	Loss of genuine segments	5, 9, 13, 17, and 21
	Presence of questionable segments	7, 11, 15, and 19
2	Reduction in antennal length	1–9
3	Displacement, loss, or addition of ring organs	1–2
4	Apical fusing of basal segments	2
5	Displacement, loss, or addition of sensory organs	2
6	Structures of unknown homology	
	Major structures	2
	Minor structures	1

in Table 7 to take into account new types of deformities reported in Warwick (1989). Step 3 was modified to include the addition of extra ring organs (see Fig. 1.42) and, as was the case for the loss of a ring organ, each additional ring organ is assessed a value of 2. Step 5 was similarly modified to include the loss or addition of sensory organs (see Fig. 1.35, 1.36). Each occurrence is assigned a base value of 2.

Index of Morphological Response

An IMR is calculated for each deformed antenna or pair of deformed antennae using the six basic steps above, with IMR representing the cumulative total of the values assessed each category of deformity, singly or in combination, as shown in Table 8. Because larvae of *Procladius* only have four antennal segments, the primary assessment for deformed antennae is increased to 5 rather than 1 as for *Chironomus*. This weighting biases the calculation, but I believe it justified as compensation in part for the overwhelming dominance of the basal segment (Segment I) in *Procladius* antennae (Warwick 1989).

The antennae in Fig. 1.35 and 1.36 differ from normalcy only by the addition of extra accessory blades (ABl). Each adds two points to the total IMR value. Similarly, the displacement of the style (St) basally on the antennae in Fig. 1.37 and 1.38 also adds two points to the total IMR value. The antenna in Fig. 1.39 lacks one segment, those segments remaining are greatly reduced in length, and the sensory organs have been displaced. In Fig. 1.40 and 1.41, the antennae have a number of structures of unknown homology, both major and minor, in addition to a number of segments lacking. The antenna in Fig. 1.42 has only one definite segment, but this is equipped with three ring organs. The additional ring organs add seven additional points to the base value of 17, two for each additional ring organ and one each because all three are displaced apically on Segment I.

Index of Severity of Antennal Deformation (ISAD) at Population and Community Levels

ISAD is interpreted as a measure of the morphological response to stress exhibited by antennae in *Procladius* populations exposed to toxicants. The index is defined as

$$(4)\quad \mathrm{ISAD} = \frac{\sum \mathrm{IMR}}{n}$$

where ΣIMR is the sum of individual IMR values for a *Procladius* population divided by n, the number of *Procladius* larvae examined in that sample or site. This represents a more precise definition of ISAD than that in Warwick (1985). There, n should have been more clearly identified as "the number of *Chironomus* specimens examined."

As in ISLD, the calculation of ISAD has been broadened to include other members of the chironomid community:

$$(5)\quad \mathrm{ISAD}_{\mathrm{Total}} = \frac{\sum \mathrm{IMR}}{N}$$

where ΣIMR is now divided by N, the total number of chironomid larvae examined in that sample site.

Eventually, indices describing the response of other structures will be included in a comprehensive measure of Population Morphological Response (PMR) for *Procladius* such that

$$(6)\quad \mathrm{PMR} = \sum_{Procladius} \mathrm{ISLD} + k_1\mathrm{ISAD} + \ldots\ldots k_x\mathrm{ISx D}$$

where x indicates other morphological structures in the head capsule or body and k is a sensitivity constant for these individual structures. By extension, this leads to the more comprehensive measure of Chironomid Community Response (CCR) where

$$(7)\quad \mathrm{CCR} = K_1\left(\sum_{Procladius} \mathrm{ISLD}_{\mathrm{Total}} + k_1\mathrm{ISAD}_{\mathrm{Total}} + \ldots k_x\mathrm{ISx D}_{\mathrm{Total}}\right) + \ldots + K_x\left(\sum_{\mathrm{Other}} \mathrm{ISx D}_{\mathrm{Total}}\right)$$

TABLE 8. Examples of estimating the Index of Morphological Response (IMR) for antennae of *Procladius*.

Fig.	Basic index number	Length reduction factor	Ring organ	Apical fusing	Sensory organs	Structures of unknown homology		Other	IMR
						Major	Minor		
1.35	5	—	—	—	2	—	—	—	7
1.36	5	—	—	—	2	—	—	—	7
1.37	5	—	—	—	2	—	—	—	7
1.38	5	—	—	—	2	—	—	—	7
1.39	7	3	—	—	2	—	—	—	12
1.40	15	—	—	—	—	—	2	—	17
1.41	15	1	—	—	2	8	—	—	26
1.42	17	3	7	—	2	—	1	—	30

TABLE 9. Frequency and severity of deformities in the ligulae of *Procladius* from precolonization (874 B.C.) to modern (1972) time in the Bay of Quinte, Lake Ontario (adapted from Warwick 1980).

Depth of sediment (cm)	Date (year)	Total number of Chironomidae (*N*)	Total number of *Procladius*		Frequency of deformities (%)		Severity of deformities		
			Number (*n*)	Deformed	*Procladius*	Total	Total IMR	ISLD	
								Procladius	Total
—	1972	201.0	48.0	1.0	2.08	0.498	2.0	0.04	0.010
0.5	1969	55.0	4.0	0	0	0	0	0	0
4.5	1951	282.5	27.0	1.0	3.70	0.354	2.0	0.07	0.007
9.5	1928	214.0	14.0	1.0	7.14	0.467	2.0	0.14	0.009
14.5	1907	367.5	26.0	1.0	3.85	0.272	2.0	0.08	0.005
19.5	1897	309.0	18.0	0	0	0	0	0	0
24.5	1888	603.5	23.0	0	0	0	0	0	0
29.5	1864	584.0	24.0	0	0	0	0	0	0
34.5	1853	522.0	29.0	0	0	0	0	0	0
39.5	1849	446.5	28.0	1.0	3.57	0.224	2.0	0.07	0.004
44.5	1835	886.5	78.0	0	0	0	0	0	0
49.5	1818	746.5	50.0	0	0	0	0	0	0
64.5	1707	1197.5	97.0	0	0	0	0	0	0
89.5	1087	1110.0	94.0	1.0	1.06	0.090	2.0	0.02	0.002
114.5	424	1486.0	118.0	0	0	0	0	0	0
139.5	238 B.C.	1133.0	115.0	1.0	0.87	0.088	2.0	0.02	0.002
163.5	874 B.C.	1112.0	102.0	0	0	0	0	0	0

where K is a sensitivity constant for individual taxa and "Other" refers to taxa other than *Procladius*. Since both indices have common denominators (n or N, respectively), each can be summed to provide a measure of response at their respective levels of biological organization.

Deformities in Stressed and Unstressed Populations of *Procladius*

Frequency and Severity of Deformities in Ligulae

Reliable background data on the frequency of deformed ligulae in unstressed communities are difficult to obtain because few contemporary environments remain untouched by contaminants of one form or another. An alternative source for such data is the subfossil record.

Warwick (1980) recorded a number of deformities in contemporary and subfossil fauna from the Bay of Quinte, Lake Ontario (Table 9). In *Procladius*, the frequency of deformed ligulae ranged from zero to 0.09% prior to the arrival of European colonists in 1784 (58.5 cm). If the average is taken for the five precolonization levels examined in detail, frequency of occurrence becomes ~0.38% in terms of the *Procladius* population, or ~0.036% in terms of the total chironomid community. Following European settlement (above 58.5 cm), frequencies increased by an order of magnitude whichever method of calculation is used.

The deformities found in the Bay of Quinte fauna represent some of the simpler types of deformities in *Procladius* ligulae. Both deformed specimens predating colonization featured modifications to the larger, outer teeth whereas those in the more recent sediments either had one less (4) or one more (6) tooth than normal, the latter occurring in the single specimen from 4.5 cm. The ISLD values presented in Table 9 suggest that, of the two indices, the community index seems to be the more reliable predictor because the values for the contemporary biocoenosis (0.010) and the subfossil thanatocoenosis (0.007) appear more comparable and the expected trends more predictable.

The trend toward increasing levels of contamination coincident with the beginning of the "chemical age" is even more apparent when the entire fauna is included. Warwick (1980) found that the frequency of deformities in *Procladius* and *Chironomus* began to increase rapidly in sediments dated after 1951 from precolonization values of 0.09% to 1.06 and 1.99% in 1972. The 1951 date closely approximates the date (1949) on which a Union Carbide facility in Belleville, Ontario, began introducing waste products, including phenols, into the bay (Warwick 1988).

In the St. Lawrence River material, frequency shows little ability to discriminate between sites unless there are very major differences in the quality of environment (Table 10). The frequencies of deformities in Lac St. Louis vary between 3.85% (Site 3) and 6.32% (Site 2) and indeed are less than the maximum value (7.14% at 9.5 cm) reported in the Bay of Quinte. Only when the very severe conditions of advanced eutrophy are added in, such as in the Laprairie basin, do frequency values rise significantly.

The values for ISLD, particularly those based on the total chironomid community, provide a much greater level of discrimination. The highest value (1.182) comes from Site 4, immediately offshore from the industrial complex at Beauharnois whereas the lowest (0.172) comes from Site 8, the area protected from the main flow of the St. Lawrence River by the island chain of Îles de la Paix.

In the Laprairie basin, the highest value (1.694) came from Site 3 instead of Site 7a or 13a. One might expect the latter sites to be more severely stressed than Site 3 by virtue of their closer proximity to the Laprairie waste treatment facility. The inversion apparent in the community response values probably is a function of this same proximity. The increasingly severe conditions imposed on the fauna by the introduction of untreated

TABLE 10. Comparison of the frequency and severity of ligula deformation in *Procladius* populations and chironomid communities from the Lac St. Louis and Laprairie basins of the St. Lawrence River.

	Lac St. Louis									Laprairie	
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 3	Site 7a
Number of larvae examined	46	253	71	368	492	441	369	704	47	62	17
Number of *Procladius* larvae	19	190	52	281	391	324	142	234	30	11	4
Number of larvae with deformed ligulae	1	12	2	15	24	18	6	14	2	3	1
Frequency of deformed ligulae (%)	5.26	6.32	3.85	5.34	6.14	5.56	4.23	5.98	6.67	27.27	25.00
Total IMR	33	214	80	435	267	215	69	121	52	105	19
Average IMR	33.00	17.83	40.00	29.00	11.13	11.94	11.50	8.64	26.00	35.00	19.00
$ISLD_{Procladius}$	1.74	1.13	1.54	1.55	0.68	0.66	0.49	0.52	1.73	9.55	4.75
$ISLD_{Total}$	0.717	0.846	1.127	1.182	0.543	0.488	0.187	0.172	1.106	1.694	1.118

TABLE 11. Comparison of the frequency and severity of ligula deformation in *Procladius* populations and chironomid communities from the Mynarski Lake and Rat Lake basins of Southern Indian Lake – Notigi Reservoir, Tobin Lake, and Port Hope Harbour, Lake Ontario.

	Central Mynarski Lake (summer)	Rat Lake (summer)	Last Mountain Lake Site I	Tobin Lake		Port Hope Harbour (B, C, E)
				Site 2	Site 17	
Number of larvae examined	734	1260	1174	1010	309	77
Number of *Procladius* larvae	76	11	619	344	97	57
Number of larvae with deformed ligulae	5	1	6	9	1	7
Frequency of deformed ligulae (%)	6.58	9.09	0.97	2.62	1.03	12.28
Total IMR	75	33	124	294	33	170
Average IMR	15.00	33.00	20.67	32.67	33.00	24.29
$ISLD_{Procladius}$	0.99	3.00	0.20	0.85	0.34	2.98
$ISLD_{Total}$	0.102	0.026	0.106	0.291	0.107	2.208

sewage has reduced the number of responding species (only three remain at Site 13a) and the population base on which to assess response. It is possible that the toxic effects of contaminants may also have been reduced by the sewage either through dilution or through complexation by "organic ligands complexing potentially-toxic heavy metals" and other contaminant sources (Whitby et al. 1976). Like most sedimentary processes, dilution and binding effects are very much dependent on rate of accumulation, and in this case, the rate is very high.

Frequency data do follow the patterns expected in Last Mountain Lake and Tobin Lake. In both cases, the sites closest to the source of contamination show higher frequencies of response (Table 11). Last Mountain Lake Site I, periodically exposed to contaminated water from the Qu'Appelle River, had a frequency of deformation in the ligula of 0.97% compared with Last Mountain Lake Site II, where the frequency was zero (not shown in Table II). Tobin Lake Site 2, directly exposed to contaminants borne by the inflowing waters of the Saskatchewan River, had a frequency of 2.62% compared with 1.03% for Tobin Lake Site 17. The ISLD values followed the same pattern with higher values of 0.106 and 0.291 for Last Mountain Lake Site I and Tobin Lake Site 2, respectively. The Last Mountain Lake data are important because the data set is large (2109 larvae) and clearly shows the subtlety of response of these larvae.

The value of the Rat Lake data are less easily interpreted because, although based on a large data set (1260 larvae), only a very small percentage (0.87%) were *Procladius*. In this case, the majority of the environmental information for this site will be provided by *Chironomus*, the dominant member of the fauna.

The level of severity of response in the fauna from the Central Mynarski Lake basin was higher than expected (Table 11); this basin is probably the least impacted by flooding and yet it has the highest ISLD value (0.102) of the five basins studied in Southern Indian Lake – Notigi Reservoir. For reasons discussed at length below, this probably represents a case where sediment–metal interactions are minimal and the availability of toxic metals to resident fauna is correspondingly high.

Both frequency of response (12.28%) and ISLD (2.208) were high in fauna from heavily contaminated Port Hope Harbour (Table 11). The fact that both values are higher in Port Hope Harbour than in Lac St. Louis Site 4, where *Chironomus* has been eliminated from the community, raises the question of the value that should be assigned for the loss of a species or taxon. The balance between *Chironomus* (24.68%) and *Procladius* (74.03%) in Port Hope Harbour is clearly abnormal and reminiscent of areas of Lac St. Louis where the balance also has been seriously altered (Warwick 1990b). But *Chironomus* is still present in Port Hope Harbour, and this fact indicates that Port Hope Harbour is the less severely

TABLE 12. Comparison of the frequency and severity of antennal deformation in *Procladius* populations and chironomid communities from the Lac St. Louis basin of the St. Lawrence River, Tobin Lake, Last Mountain Lake, and Port Hope Harbour, Lake Ontario.

	Lac St. Louis						Tobin Lake	Last Mountain Lake		Central Mynarski Lake
	Site 2	Site 4	Site 5	Site 6	Site 7	Site 8	Site 2	Site I	Site II	
Number of larvae examined	253	368	492	441	369	704	1010	1174	935	734
Number of *Procladius* larvae	190	281	391	324	142	234	344	619	93	76
Number of larvae with deformed antennae	2	6	7	4	1	5	3	2	1	1
Frequency of deformed antennae (%)	1.05	2.14	1.79	1.23	0.70	2.14	0.87	0.32	1.08	1.32
Total IMR	32	65	51	34	11	64	36	14	7	9
Average IMR	16.00	10.83	7.29	8.50	11.00	12.80	12.00	7.00	7.00	9.00
$ISAD_{Procladius}$	0.17	0.23	0.13	0.10	0.08	0.27	0.10	0.02	.08	0.12
$ISAD_{Total}$	0.127	0.177	0.104	0.077	0.030	0.091	0.036	0.012	0.008	0.012

TABLE 13. Interbasin comparisons of Indices of Contamination (I_c) based on overlapping combinations of trace metals (according to Champoux and Sloterdijk 1988). Underlined values indicate indices based on total number of metals analysed. Accompanying values not underlined indicate indices based on that subset of metals.

	Lac St. Louis							Southern Indian Lake – Notigi Reservoir							
Trace metal combinations	L-12 (near Site 2)	L-21 (ca. Sites 3–6)	L-2 (Site 7)	L-6 (Site 8)	Laprairie basin Site 3	Laprairie basin Site 7	Laprairie basin Site 13	Rat Lake Spring	Rat Lake Summer	C. Mynarski Lake (summer)	South Bay (summer)	Wupaw Bay (summer)	Tobin Lake Site 2	Tobin Lake Site 17	Port Hope Harbour
Zn, Cu, Pb, Ni, Hg, As, Cd, Co, Cr			1.86	1.81									<u>0.80</u>	<u>1.00</u>	
Zn, Cu, Pb, Ni, Hg, As, Cd	<u>1.99</u>	<u>5.22</u>	<u>1.81</u>	<u>1.98</u>									0.49	0.79	
Zn, Cu, Pb, Ni, Hg Cr			2.38	2.43	<u>1.37</u>	<u>2.27</u>	<u>3.42</u>						0.97	1.26	
Zn, Cu, Pb, Ni, Hg	2.28	4.19	2.12	2.43	1.22	2.12	3.50						0.45	0.84	
Zn, Cu, Pb, Ni	2.48	3.36	1.50	1.21	1.45	2.03	2.06						0.50	1.01	4.73
Zn, Cu, Pb, Ni, Cr			1.93	1.45	1.59	2.23	2.30						1.11	1.48	4.18
Zn, Cu, Pb, Ni, Cd, Co, Cr			1.63	1.19									0.85	1.14	<u>3.32</u>
Zn, Cu, Hg, Cd, Co			1.94	2.28				<u>0.44</u>	<u>0.94</u>	<u>1.22</u>	<u>0.78</u>	<u>0.70</u>	0.39	0.57	
Zn, Cu	3.21	4.71	1.70	1.51	1.25	2.02	2.78	0.73	0.74	1.20	0.81	0.53	0.63	1.06	3.47

contaminated site, in contradiction to the numerical indices. The question then becomes "What value do we assess the loss of a life form?" As discussed below, this has important ramifications for interpretation of the bell-shaped response curve of fauna and communities to contaminants.

Frequency and Severity of Deformities in Antennae

In terms of the total chironomid community, comparatively uncontaminated Last Mountain Lake Site II had the lowest value for ISAD (0.008) recorded for *Procladius* (Table 12). This probably is the best estimate of the background level of antennal deformation in contemporary *Procladius* fauna available at the present time. The value for Site I (0.012) was 1.5 times higher and reflects the pattern of contamination expected in Last Mountain Lake both from ISLD and other environmental considerations. This same pattern was not reflected either in frequency of deformed antennae or in terms of the *Procladius* population, however. Why this should be so is not immediately apparent, but, as discussed below, it probably reflects differences in the availability of toxicants in different sedimentary environments. At the other end of the spectrum, the highest value for ISAD came from Lac St. Louis Site 4, the site immediately offshore from the industrial complex at Beauharnois.

Deformities, Contaminants, and Environmental Interactions

A number of difficulties become apparent when trying to correlate the effects of contaminants on field populations at this stage of development. One difficulty is that the biological and chemical data sets represent incomplete measures of cause and effect. The biological data used here are based only on the subset for antennae and ligula in *Procladius*. Whereas these two structures probably account for the greater part of community response in the Lac St. Louis study, responses in other head capsule structures (paraligulae (excluding fused paraligulae), hypopharyngeal pecten, dorsomenta, mandibles, etc.) undoubtedly will modify and contribute to the overall interpretation of response in *Procladius*. In addition, the contributions of other genera, although they may be limited in terms of the numbers of individuals actually present (Warwick 1990b), may become very significant if their response rates reflect rare or

TABLE 14. Chironomid Community Response (CCR) to contaminants based on deformities in the ligulae and antennae of *Procladius*. (— = no data)

	CCR			I_c		
				Trace metals		
	ISLD	ISAD	*Procladius* subset	All available (no.)	Zn, Cu (subset)	Organics
St. Lawrence River						
Lac St. Louis Site 1	0.717	0	0.717	—	—	—
Lac St. Louis Site 2	0.846	0.127	0.973	1.99(7)	3.21	3.65
Lac St. Louis Site 3	1.127	0	1.127			
Lac St. Louis Site 4	1.182	0.177	1.359	5.22(7)	4.71	20.33
Lac St. Louis Site 5	0.543	0.104	0.647			
Lac St. Louis Site 6	0.488	0.077	0.565			
Lac St. Louis Site 7	0.187	0.030	0.217	1.81(9)	1.70	2.90
Lac St. Louis Site 8	0.172	0.091	0.263	1.98(9)	1.51	2.50
Lac St. Louis Site 9	1.106	0	1.106	—	—	—
Laprairie basin Site 3	1.694	0	1.694	1.37(6)	1.25	—
Laprairie basin Site 7a	1.118	0	1.118	2.27(6)	2.02	—
Laprairie basin Site 13a	0	0	0	3.42(6)	2.78	—
Southern Indian Lake – Notigi Reservoir						
Wupaw Bay	0	0	0	0.70(5)	0.53	—
South Bay	0	0	0	0.78(5)	0.81	—
Central Mynarski Lake	0.102	0.012	0.114	1.22(5)	1.20	—
Rat Lake (spring)	0	0	0	0.44(5)	0.73	—
Rat Lake (summer)	0.026	0	0.026	0.94(5)	0.74	—
Last Mountain Lake Site I	0.106	0.012	0.118	—	—	—
Last Mountain Lake Site II	0	0.008	0.008	—	—	—
Tobin Lake Site 2	0.291	0.036	0.327	0.80(9)	0.63	—
Tobin Lake Site 17	0.107	0	0.107	1.00(9)	1.06	—
Tobin Lake Site V	0	0	0	—	—	—
Tobin Lake Site VI	0	0	0	—	—	—
Port Hope Harbour (Sites, B, C. E)	2.208	0	2.208	3.32(7)	3.47	—

endangered species living near or at the limit of their ecological (Saether 1970) or tolerance (Warwick 1990b) ranges. When these limits are exceeded, loss or elimination follows (Warwick 1990c). Loss is a difficult parameter to quantify, but this information is vital to any equation if the true impact of contaminant effects is to be estimated.

The chemical data presented here also are incomplete. This is certainly evident in Table 13 where only two metals, copper and zinc, are common to all four studies. In most cases, the elements and compounds measured were determined more by economics, available technology, and the level of knowledge and concern than any other factor. Beyond this, and even of more concern and the reason underlying the recent shift towards biological monitoring, lies the fact that it is almost impossible to break down, identify, and measure the component compounds making up the complex contaminant burden affecting many environments. There are simply too many toxic compounds entering the environment, too many degradation products, too many products of synergistic or antagonistic combination, and too many environmentally modified effects to be analyzed efficiently and effectively.

Despite these limitations, and they must be acknowledged, comparison of the chemical and biological data shows (Table 14) some very interesting correlations. At first glance, the most obvious are the data for Port Hope Harbour where the values for the subset of CCR based on *Procladius* and the metals-based I_c are both correspondingly high. In the latter case, the index probably would be considerably higher if criteria were available to assess the radionuclide contribution to the toxic burden to which these fauna were exposed.

Secondly, and also obvious, are the very high metals- and organics-based I_c's for the area immediately offshore from the industrial complex at Beauharnois. The highest CCR value is from Lac St. Louis Site 4, the site closest (~100 m) to the factory complex. Although this value is not as high as that for Port Hope Harbour, a number of the major components of the chironomid community, particularly the genus *Chironomus*, have been eliminated from the contemporary fauna. Analysis of subfossil assemblages shows that *Chironomus* previously was an important component (~45%) of the community at Site 4 (Warwick 1990b). If its loss and the loss of a number of other genera could be evaluated, total CCR for Site 4 probably would be much higher than for Port Hope Harbour, where *Chironomus* is still present, albeit in reduced numbers.

On a more subtle level, both I_c and CCR show (Table 14) that the Central Mynarski Lake basin is the more heavily impacted of the Southern Indian Lake – Notigi Reservoir basins studied. In this case, the sedimentary milieu seems to be responsible for the significant responses shown by both faunal and chemical indices. According to Bodaly et al. (1984a, 1984b), the amount of new mercury entering a basin is determined by the extent of areal flooding. However, in terms of

flooding effect, this basin is among the least impacted of the reservoir. The area flooded to provide new sources of toxic elements and the amount of erosion resulting from water level changes were both minimal. On the other hand, the sediments at this site are highly organic and conducive to bacterial mobilization processes which directly affect the availability of trace metal elements (Rudd et al. 1983). Under such conditions, toxic elements like mercury probably are readily available to the fauna, and this is reflected in the higher CCR value.

The converse is true in South Bay where wind stress is high, shoreline erosion extensive, and sedimentation/turbidity effects pronounced. Following the Bodaly et al. (1984a, 1984b) hypothesis, South Bay should be heavily impacted and yet no morphological response was detected in either the ligulae or antennae of *Procladius*, even though the genus made up almost 50% of the fauna collected. Contaminants simply do not appear to be available to the genus in environments where turbidity is high and exposure minimized through binding, burial, and the interruption of bacterially mediated mobilization processes by the accumulation of sterile mineral sediment. Rudd and Turner (1983) also demonstrated this phenomenon in Southern Indian Lake where additions of uncontaminated clay-rich sediment to their experimental enclosures impeded bacterial methylation and reduced the availability of mercury to fish and other biota by an order of magnitude (Rudd et al. 1983).

The effects of availability also are evident in the seasonal data from the Rat Lake basin where both I_c and CCR increased during the summer months (Table 14). Methylation of mercury is known to increase during summer when water temperatures rise and bacterial activity increases (Ramsey and Ramlal 1986), and similar mobilization processes probably affect the availability of other contaminant elements as well. If the biological increases do reflect a response to this greater availability, then the chironomid community is sensitive indeed to very subtle changes in its environment.

The effect of mineral sediment accumulation is evident again in the comparison of the two Tobin Lake sites (Table 14). Site 2, by virtue of its greater exposure, should be the more heavily impacted of the two sites. It is directly exposed to the full contaminant burden of the Saskatchewan River inflow whereas Site 17, located in a flooded backwater area of the reservoir, is only indirectly exposed. This relationship is correctly shown by ISLD and ISAD for *Procladius*. The respective values were 0.291 and 0.036 for Site 2 compared with 0.107 and zero for Site 17. The values for I_c were reversed, however, with the value for Site 2 (0.80) lower than that (1.00) for Site 17. The inversion may again be a product of mineral sediment dilution. Site 2 is located in the delta area of the reservoir where the rate of mineral sediment accumulation is very high. Although Site 17 is exposed to a certain amount of redeposition of mineral sediments as a result of reservoir drawdown and water level regulation (and there is some evidence that mineral sediments have affected contaminant availability and biological response at this site), its rate of accumulation is orders of magnitude less than Site 2. Sediment dilution is effected both qualitatively and quantitatively, and the fact that the index for Site 2 was not further depressed probably is indicative of the high contaminant loadings borne by the river. Without the appropriate biological data and some knowledge of sedimentation patterns, simple chemical indices like I_c could be misleading and result in conclusions concerning the respective states of contamination of the two sites that were not correct. These data also raise the question "Just how far can the effects of contamination be depressed by mineral sediment accumulation and how can this effect be measured?"

The same processes probably operate in Last Mountain Lake as well. Site I is exposed to contaminants from the Qu'Appelle River, and ISLD and ISAD both identify it as the more contaminated site (Table 14). The sediments at Site I are more highly mineralized than those at Site II, however. The site is much shallower (3.9 m) than Site II (23.1 m) and more exposed to wind stress, erosion from beach areas, and resuspension of bottom sediments. Although no chemical data are available for comparison, the possibility that mineral sediments may be suppressing contaminant effects at Site I suggests that there may be a real need for further investigation of this habitat.

Lac St. Louis Site 7 also appears to be affected by the accumulation of mineral sediments. This site receives sedimentary materials from the nearby Rivière St. Louis (Germain and Janson 1984) in contrast with neighbouring Site 8 which is largely shielded from exposure to mineral sediment inputs and contaminants (including mercury from the chloralkali plant upriver at Beauharnois) by the island complex of Îles de la Paix. Site 7 is the more heavily impacted site, and this is correctly shown by ISLD with values of 0.187 and 0.172 for Sites 7 and 8, respectively. Like Tobin Lake, ISAD values showed the reverse order, however, with values of 0.030 and 0.091 for Sites 7 and 8, respectively. The interpretation of antennal response is very much determined by the side of the bell-shaped dose–response curve on which any particular datum falls (Warwick 1985). In this instance, the inversion suggests that the data fall on the right-hand side of the dose–response curve, that is, the side of the curve indicating greater contamination. This decision is supported by the fact that both sites support only remnant populations of *Chironomus*, neither of which displays deformities in their antennae. This is analogous to the Port Hope Harbour situation where, despite a multiplicity of deformities in all other parts of the head capsule in more than 85% of the *Chironomus* population, there were no deformed antennae in fauna from this otherwise heavily contaminated site (Warwick 1988). If this dichotomy is correct, the decision to treat ISLD and ISAD separately implies an independence of operation and suggests that the two indices may not be directly additive as shown in Table 14, but require the compensatory constant suggested in equation 6 to adjust for differences in response.

Tobin Lake Sites V and VI display similar reverse order. Site VI should be the more highly contaminated of the two sites because it is located towards the headstock of the Squaw Rapids dam in the deepest parts of the reservoir in an area of low hydraulic energy and a natural sink for organic-bound, contaminant-laden materials (Warwick 1985). These interpretations are substantiated both by the presence of large quantities of woodpulp fibriles in the sediments and by the presence of massively deformed larvae. Despite the biological evidence and hydraulic energy considerations, ISAD values for *Chironomus* are only 1.193 at Site VI compared with 1.632 at Site V. However, when these data are placed on the right-hand side of the dose–response curve, they become consistent with the field observations and biological evidence.

Few studies of living systems have demonstrated the complete spectrum of response to a given environmental stressor; most cover only portions of the response curve (Lugo 1978). Kosalwat and Knight (1987) and Van de Guchte and Van Urk (1989) demonstrated the initial, positive stage of the response curve using chironomid larvae exposed to increasing concentrations of copper, cadmium, and zinc. In both studies, response

was confined to the epipharyngeal pecten and was significantly correlated with the concentrations of metals employed. At the other end of the spectrum, Warwick (1985) demonstrated the final, negative stages of the response curve in larvae exposed to increasing concentrations of DDE. In this study, the frequency and severity of deformities in the antennae of *Chironomus tentans* both declined as the concentration of pesticide increased. Significantly, the trend toward extinction of response indicates that the antennae of *Chironomus* are much more sensitive to DDE than the initial concentrations of 1 μg DDE·L^{-1} used in the experiment (Warwick 1985).

At present, there are no examples available from studies of chironomid morphology to demonstrate Lugo's (1978) condition of steady state, but Frank and Köhn (1982) demonstrated that response can be reversed if the stressor is removed. These authors removed larvae of *Chironomus riparius* and *C. plumosus* from the highly polluted Teltowkanal (Berlin) and reared them under clean conditions in the laboratory. Frequencies of deformities, which in both populations exceeded 40% in the canal (Köhn and Frank 1980), declined to between zero and 2% after one generation in clean laboratory water.

Response curves function at a number of different levels of biological organization. Response curves illustrated by other ecologists and toxicologists (Odum 1971, 1981; Odum and Kroodsma 1976; Odum et al. 1979; Regier and Cowell 1972; Gibbons 1976; Chan et al. 1984; Cairns and Niederlehner 1989) differ in shape and style, and so probably do those representing the responses of individual structures (Warwick 1990a). Larval antennae appear to respond to contaminants at the level of the organ rather than at the level of the individual because antennal deformities and deformities in the remaining structures of the head capsule seemingly act independently. The absence of deformed antennae in larvae from highly contaminated sites like the Teltowkanal and Port Hope Harbour are excellent examples of the negative or extinction phase of the response curve for larval antennae.

At the community level of organization, the loss of taxa from highly contaminated sites like Site 4 in the St. Lawrence River also demonstrates the negative or extinction phase. Without the contribution of responding species, or in the absence of ways to compensate for their loss, the index must automatically decline. The closest example to the condition of steady state at the community level probably is the Port Hope Harbour site. Compared with Site 4 in the St. Lawrence, *Chironomus* larvae are still represented at the site, but the ratio expected between this taxon and *Procladius* is not in balance. This suggests that the *Chironomus* population might be nearing the point of collapse and the beginning of elimination.

At each level of organization, three primary processes appear to operate (Fig. 2) to determine response in bottom-dwelling biological communities: (1) the contamination process, which represents the input or supply side of the equation, and the (2) trophic and (3) sedimentation processes, which determine the availability side of the equation. The interaction between supply and availability is then expressed in terms of faunal response at any one or more levels of biological organization. Contamination encompasses not only the input of chemicals, but also their by-products, degradation products, and the products of synergistic and antagonistic interaction. The availability of these products to bottom-dwelling communities is determined by the sedimentary milieu into which they are deposited. Under normal circumstances, trophic state in large measure determines the ratio of organic and mineral components in sediments

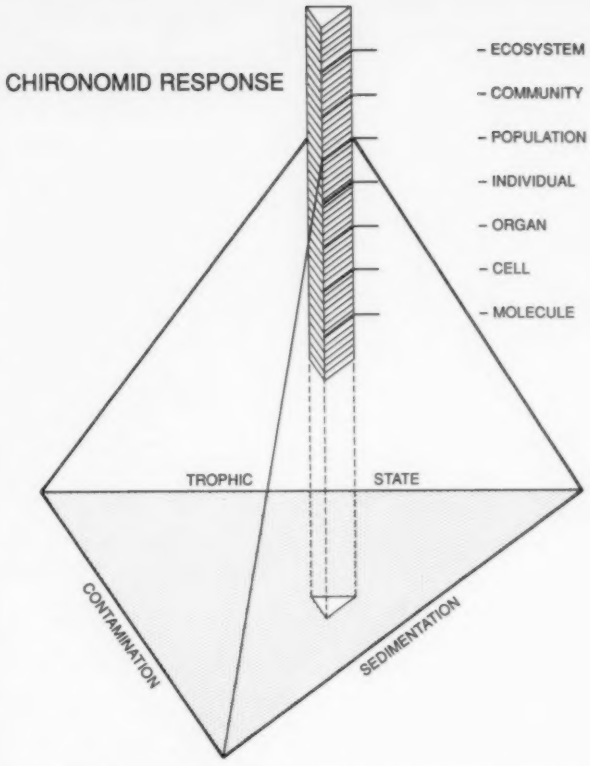

FIG. 2. Diagrammatic interpretation of the interaction between the major processes of contamination, trophic state, and sedimentation and biological response at different levels of biological organization. As indicated here, response is shown at the level of the population.

which in turn govern binding and remobilization processes. However, under abnormal circumstances where rates of accumulation become excessive, these processes are modified or halted completely; for example, even minor amounts of contaminant may have a major impact where rates of mineral sediment accumulation are low (e.g. the Mynarski Lake basin or Tobin Lake Site 17), but where rates are high, the effects of major inputs of contaminants may be ameliorated through burial (Tobin Lake Site 2, the South Bay basin, or Lac St. Louis Site 7) or through interference with bacterial remobilization processes (the Rat Lake basin or the Bay of Quinte) by mineral sediment accumulation. The Laprairie basin represents a case where the abnormally high rates of accumulation of organic materials may have masked the effects of contaminants either through elimination of the resident fauna by the reduction of dissolved oxygen supplies or by reduction of the contaminant burden through complexation and/or dilution. In conclusion, the complex interaction of all these processes must be carefully weighed before the impact of contaminants can be assessed for a given aquatic ecosystem.

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Orientation des déplacements de crabes des neiges mâles (*Chionoecetes opilio*) marqués dans le sud-ouest du golfe du Saint-Laurent

Louise Lefebvre[1] et Jean-Claude F. Brêthes

Centre océanographique de Rimouski, Département d'océanographie, Université du Québec à Rimouski, Rimouski (Québec) G5L 3A1, Canada

Lefebvre, L. et J.-C. Brêthes. 1991. Orientation des déplacements de crabes des neiges mâles (*Chionoecetes opilio*) marqués dans le sud-ouest du golfe du Saint-Laurent. Can. J. Fish. Aquat. Sci. 48: 1167–1175.

Un total de 7 760 *Chionoecetes opilio* mâles ont été marqués et relachés au niveau du fond à l'entrée de la baie des Chaleurs et à proximité des îles de la Madeleine, dans le sud-ouest du golfe du Saint-Laurent, en 1986 et 1987. Sur ce nombre, 976 individus, tous morphométriquement matures, ont été récupérés entre 1987 et 1989. La distance moyenne parcourue est de 9,6 km pour la baie des Chaleurs (maximum 47,0 km) et de 13,7 km pour les îles de la Madeleine (maximum 89,5 km). La taille n'a aucun effet significatif ni sur la distance parcourue ni sur l'orientation des déplacements. Les individus relachés sur des niveaux bathymétriques supérieurs ou intermédiaires suivent le gradient de pente descendant. Dans le secteur de la baie des Chaleurs, les crabes de profondeur montrent une tendance à remonter le pente. Vers les îles de la Madeleine, les individus relachés en profondeur restent majoritairement dans l'axe de la vallée, mais une tendance à la remontée existe également. Il semblerait exister deux types de mouvements, soit un mouvement général descendant, perturbé par des déplacements saisonniers, sans doute liés à la reproduction. Des déplacements de grande amplitude semblent indiquer que des contacts entre sous-stocks locaux peuvent exister.

A total of 7760 *Chionoecetes opilio* males were marked and released on the bottom at the entrance to Chaleur Bay and near the Îles-de-la-Madeleine in the southwestern Gulf of St. Lawrence in 1986 and 1987. Of this number, 976 individuals, all morphometrically mature, were recovered between 1987 and 1989. The average distance covered was 9.6 km for Chaleur Bay (maximum 47.0 km) and 13.7 km for the Îles-de-la-Madeleine (maximum 89.5 km). Size had no significant effect on distance covered or direction of movement. Individuals released at higher or intermediate bottom depths followed the slope downward. In the Chaleur Bay sector, the crabs at greater depth showed a tendency to move up the slope. Near the Îles-de-la-Madeleine, most of the individuals released at greater depth stayed on the valley floor, but there was also a tendency to move upward. It appears that there are two types of travel, a general downward movement, disturbed by seasonal shifts which are undoubtedly related to reproduction. Large movements suggest the possible existence of contacts between local substocks.

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L'exploitation du crabe des neiges (*Chionoecetes opilio*) dans l'est du Canada est administrativement divisée en pêche semi-hauturière et pêche côtière. Cette distinction provient de l'hypothèse que peu d'interactions existent entre ces deux régions (Brêthes et Coulombe 1987). Toutefois cette supposition n'est certainement pas respectée pendant la durée des stades larvaires pélagiques qui pourrait créer un certain niveau de mélange génétique entre les présentes unités administratives (Davidson *et al.* 1985). Des expériences de marquage de mâles *Chionoecetes opilio* menées par Watson (1970) et Watson et Wells (1972), sur les fonds de pêche, ont conduit à la conclusion de mouvements de petites amplitudes, généralement moins de 20 km, même si quelques déplacements supérieurs à 50 km ont été observés sur une période de 3 ans. Ces observations suggérèrent que peu d'échanges existent entre les zones de pêche. Selon cette conclusion et sur la base d'émergence d'industries régionales, les premières zones de pêche côtière ont été ouvertes en 1977 (Elner 1982). Dans ce contexte, une bonne connaissance des mouvements du crabe des neiges devient indispensable à la saine gestion de cette pêcherie.

Des études directes ou indirectes ont permis de mieux connaître les déplacements à long terme. Dans l'Atlantique canadien, Taylor *et al.* (1985) et Hooper (1986) ont posé l'hypothèse d'une migration de reproduction au printemps, vers les fonds peu profonds, à Bonne Bay (Terre-Neuve). Plus récemment, Comeau *et al.* (1989) ont montré que ce déplacement ascendant concerne principalement les plus petits individus matures en raison de phénomènes d'exclusion, liés à des comportements agressifs, et de la géomorphologie des bassins. Dans le Pacifique nord, McBride (1982) a observé des mouvements à larges échelles couvrant une distance moyenne de 79 km. Des informations indirectes sur les mouvements des espèces peuvent être déduites de l'étude des distributions de fréquence de taille. Coulombe *et al.* (1985) ont suggéré que les crabes pouvaient se déplacer en des eaux plus profondes suivant leur maturité morphométrique associée à l'augmentation de leur taille. Bouchard *et al.* (1986) émettent l'hypothèse que le recrutement au stock de pêche est du type "peloton" (Ricker 1980), les individus se déplaçant vers les fonds de pêche profonds en provenance des niveaux bathymétriques supérieurs

[1]Adresse actuelle : Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, (Québec) G5H 3Z4, Canada.

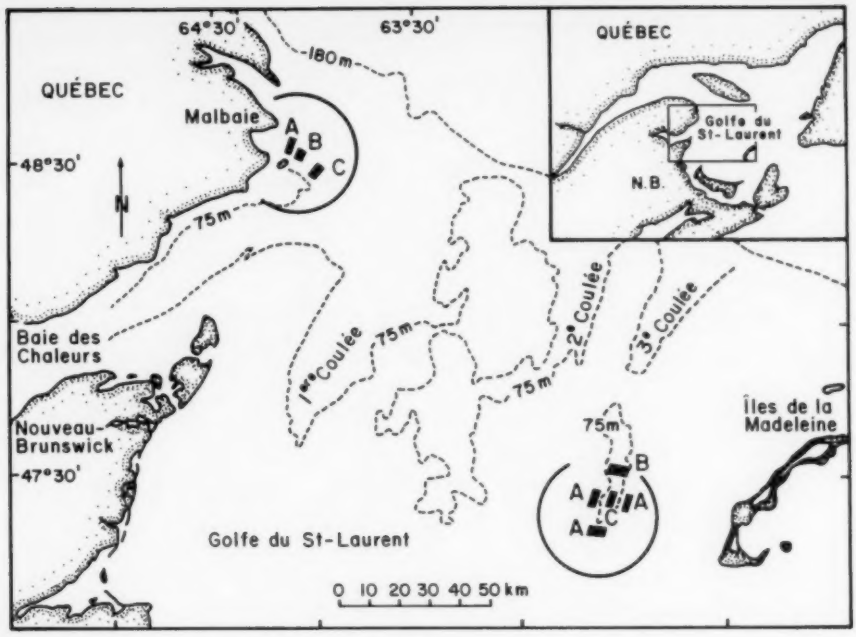

FIG. 1. Localisation de l'aire d'étude et position des zones de marquage (A : zone peu profonde; B : zone intermédiaire; C : zone profonde).

selon un processus de déplacement, dépendant de la taille des individus impliqués. Cette suggestion est appuyée par les données de Coulombe (1984) qui a observé des concentrations de crabes de taille commerciale sur des zones soumises à de faibles taux d'exploitation. Ces régions ont été qualifiées de « zones-tampons », susceptibles de compenser l'abondance décroissante du crabe des neiges sur les fonds de pêche selon un mécanisme de régulation de densité.

Selon les précédentes suppositions, les crabes localisés sur les fonds moins profonds pourraient présenter un mouvement orienté vers des niveaux bathymétriques inférieurs, mouvement qui ne devrait pas être observé pour les individus localisés en profondeur. Sur ces hauts-fonds, les plus gros individus devraient démontrer une orientation résiduelle plus évidente que les plus petits. Un autre point d'intérêt demeure la possibilité d'échange entre les fonds de pêche voisins, malgré des barrières géographiques évidentes. Ce travail, présentant des résultats sur des expériences de marquage dans le sud-ouest du golfe du St-Laurent, tente d'apporter de nouvelles réponses à ces interrogations.

Matériel et méthodes

Marquage et recapture

Les campagnes de marquage ont été entreprises dans le sud-ouest du golfe du St-Laurent (fig. 1) durant l'automne 1986 et l'été 1987. Deux secteurs ont été choisis : la Malbaie, à l'entrée de la baie des Chaleurs et une vallée à l'ouest des îles de la Madeleine (« troisième coulée »). Ces zones présentent un gradient de profondeur; les niveaux profonds sont exploités et les secteurs les moins profonds correspondent à des zones-tampons telles que définies par Coulombe (1984).

Les crabes sont marqués à l'aide d'une attache de type « spaghetti » (Fujita et Takeshita 1979) numérotée, nouée autour de la carapace entre les pattes marcheuses. Cette marque est facile d'utilisation et facilement repérable lors de la capture. Elle ne lèse pas l'animal, mais elle présente l'inconvénient d'être perdue lors de la mue. Du fait que nous étudions des déplacements sur une période relativement brève (1 à 2 ans) et sur des crabes morphométriquement matures, et donc qui ne sont plus susceptibles de muer (Conan et Comeau 1986), cet inconvénient est relativement limité.

Les individus sont capturés au casier conique à maille étirée de 131 mm (maille commerciale) pour la région des îles de la Madeleine et de 45 mm pour la région de la baie des Chaleurs. Tous les casiers sont appâtés au hareng et relevés après une nuit d'immersion. La largeur maximale de la carapace (LC) est mesurée au millimètre près, ainsi que la hauteur de la pince pour vérifier la maturité morphométrique. Les crabes sont marqués et relâchés, moins de 1 h après leur capture, au niveau du fond à l'aide de la cage décrite par Farmer et Al-Attar (1981), pour éviter les dispersions par les courants et ainsi connaître avec précision le site de largage. Lorsque le soleil était trop ardent, les opérations de marquage se déroulaient dans la cale réfrigérée.

Etant donné que la pêche commerciale ne permet de recueillir que les crabes mâles, l'étude ne porte que sur le déplacement de ceux-ci. Toutefois, lors de la campagne à l'entrée de la baie des Chaleurs, une série de femelles a été marquée.

Les résultats de retour (étiquette, date et position de recapture) sont fournis par les pêcheurs qui recevaient une prime en échange des informations transmises. Beaucoup de ces informations ont été récoltées par les agents du ministère des Pêches et des Océans du Canada, région du Golfe. Les pêcheurs qui ont fait parvenir des renseignements douteux ont été contactés par téléphone afin de vérifier leurs données. Par la suite, un compte-rendu sur le parcours des crabes était communiqué.

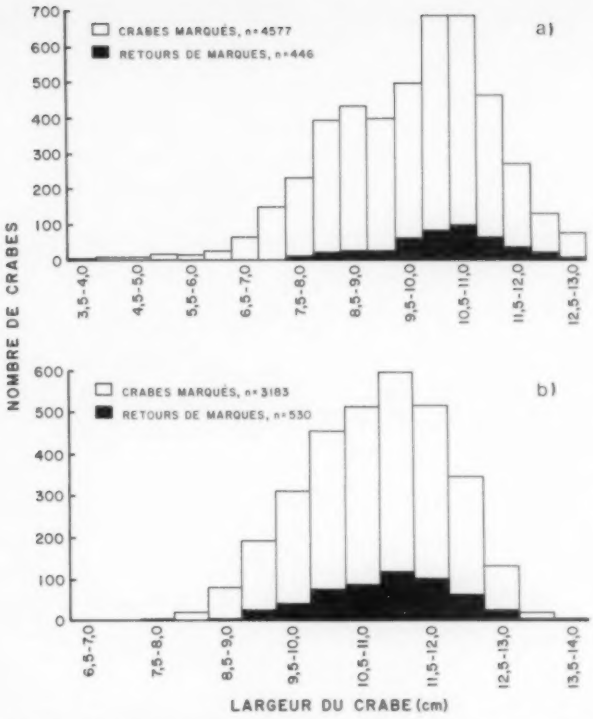

FIG. 2. Distribution des fréquences de taille des *C. opilio* mâles marqués et recapturés dans la région a) de la baie des Chaleurs et b) des îles de la Madeleine.

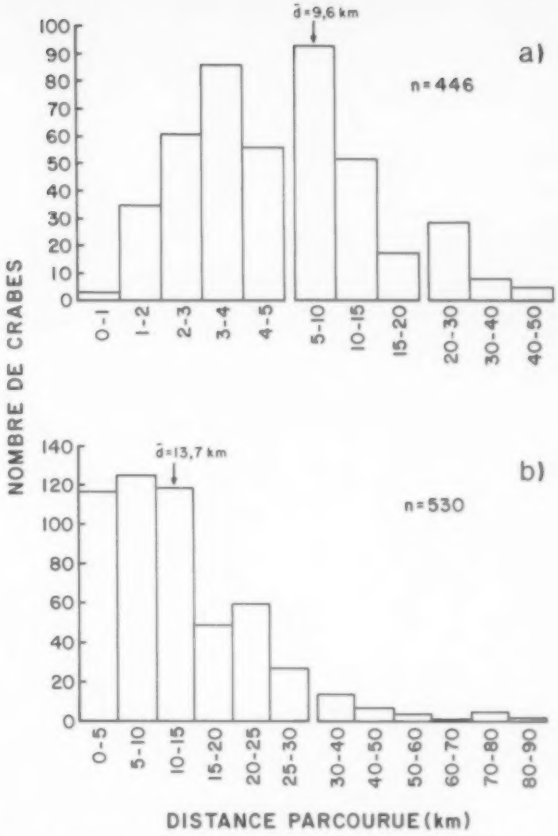

FIG. 3. Distances parcourues (après 1 ou 2 ans de liberté) par les *C. opilio* mâles marqués dans la région a) de la baie des Chaleurs et b) des îles de la Madeleine.

Des retours de marques proviennent de campagnes antérieures (1983, 1984 et 1985), effectuées à d'autres fins.

Analyse des données de recapture

Les positions de recapture soumis en lecture Loran-C sont transformées en données latitude–longitude par un logiciel fourni par le ministère des Pêches et des Océans du Canada (R. Dufour, Institut Maurice-Lamontagne, Mont-Joli (Québec), comm. pers.). Puis, pour chaque crabe, les positions de marquage et de recapture de coordonnées rectangulaires sont transformées en coordonnées polaires afin d'obtenir la distance d parcourue par le crabe et son orientation $\theta°$ par rapport au nord géographique.

Pour les fins de l'analyse, afin de mieux cerner l'effet de la taille des crabes sur les déplacements, ceux-ci sont regroupés selon trois classes de LC : 75 à 94 (non-recrutés à la pêcherie, 95 à 114 et 115 à 130 mm. Cette subdivision correspond sensiblement à la structure de la distribution des fréquences de taille observées (fig. 2). Pour examiner l'influence de la profondeur, les secteurs de marquage ont été regroupés en trois zones distinctes par région, selon la bathymétrie des lieux. A l'entrée de la baie des Chaleurs, la profondeur augmente avec l'éloignement de la côte et le regroupement est effectué comme suit : < 94, 94 à 105 et > 105 m. Aux îles de la Madeleine, une zone peu profonde (< 70 m) est constituée de trois secteurs de marquage encadrant une coulée (fig. 1). Tous les calculs sur les orientations dans cette zone ont été corrigés de façon à ce que l'effet de la pente soit annulé. Pour chaque crabe marqué sur la pente donnant vers l'est, une rotation de 270° est ajoutée à leur orientation et pour ceux de la pente vers l'ouest, une rotation de 90° est additionnée. La direction $\theta°$ au lieu d'être le nord géographique, devient la direction descendante de la pente (vers la coulée). Dans la coulée se situe la zone intermédiaire (70 à 81 m) et une zone profonde (> 81 m).

On cherche à savoir si la taille des crabes dans chacune des zones de profondeur influence les déplacements. Les comparaisons des distances moyennes parcourues sont étudiées à l'aide d'une analyse de variance à un critère de classification (ANOVA) selon la procédure du logiciel SPSS^x. Pour l'étude des orientations, pour chaque classe de taille dans chaque zone de profondeur, un test non-paramétrique de Rayleigh (Batschelet 1981) est d'abord effectué. Cette méthode vérifie si la population dans laquelle l'échantillon a été prélevé possède une direction moyenne (distribution circulaire non-uniforme). Le test de Rayleigh ne considère que les directions individuelles de déplacement, θ_i, et suppose que toutes les distances parcourues $d_i = 1$. Le test z de Rayleigh est utilisé pour vérifier l'hypothèse nulle (H_0 = population sans direction moyenne). Le résultat est comparé aux valeurs critiques tabulées (Zar 1984). Toutefois, ce test requiert l'unimodalité de la distribution et l'on doublera la valeur des angles si la distribution s'avère bimodale (Zar 1984).

Lorsque les distributions démontrent une orientation significative, les angles moyens des crabes de classe de taille dif-

TABLEAU 1. Résultats du test z de Rayleigh sur les orientations prises par les *C. opilio* mâles marqués à l'entrée de la baie des Chaleurs et dans la région des îles de la madeleine (n = nombre de recaptures; θ : angle moyen; r : longueur résiduelle moyenne).

Profondeur (m)	LC (mm)	n	θ (degrés)	r	z	Prob. z
			Baie des Chaleurs			
	< 95	20	162,2	0,712	10,17	< 0,001
<94	95 à 114	32	167,4	0,514	8,46	< 0,001
	⩾ 115	3	210,4	0,586	1,03	n trop faible
	< 95	21	157,8	0,667	9,36	< 0,001
94 à 105	95 à 114	128	166,9	0,524	35,19	< 0,001
	⩾ 115	28	144,3	0,624	10,92	< 0,001
	< 95	47	133,7[a]	0,361	6,16	< 0,01
> 105	95 à 115	140	358,0	0,933	124,20	< 0,001
	⩾ 115	27	186,9	0,388	4,07	< 0,02
			Îles de la Madeleine			
	< 95	17	6,9	0,610	6,33	< 0,001
< 70[b]	95 à 114	156	24,1	0,465	33,86	< 0,001
	⩾ 115	86	37,6	0,477	19,61	< 0,001
	< 95	4	124,4	0,688	1,89	n trop faible
70 à 81	95 à 114	36	56,8	0,467	7,87	< 0,001
	⩾ 115	15	42,3[a]	0,560	4,71	< 0,01
	< 95	15	50,8	0,139	0,29	> 0,5
> 81	95 à 115	149	58,9	0,207	6,41	< 0,002
	⩾ 115	52	10,3[a]	0,480	11,99	< 0,001

[a]Distribution bimodale : les angles ont été doublés.
[b]Données corrigées selon la pente.

férente pour chaque zone de profondeur peuvent ensuite être comparés à l'aide du test de Watson–Williams généralisé pour échantillons multiples pour vérifier $H_0 : \mu_1 = \mu_2 = \ldots = \mu_k$. Le test de Watson–Williams est paramétrique et assume que chacun des échantillons provient d'une population de distribution circulaire normale et de dispersions semblables. Heureusement, le test est robuste aux écarts des conditions (Zar 1984), mais lorsque les distributions ne sont pas unimodales, il est recommandé d'utiliser le test U^2 de Watson qui compare les distributions deux à deux.

Pour vérifier si une différence existe dans les déplacements des crabes entre les zones de profondeur, les mêmes tests ont été utilisés sur les distances et les orientations, mais seulement pour la classe de taille dominante (95 ⩽LC < 115 mm) afin d'éliminer un éventuel effet de la taille des individus. Pour cette classe de taille, on calcule les vecteurs résultants (de longueur D et d'angle Γ) pour chaque zone de profondeur en tenant compte cette fois, de la distance d et de l'orientation θ^0 de chaque crabe. Si une population est considérée se déplacer au hasard, ou pas du tout, les distances et directions individuelles de déplacement devraient, en moyenne, s'annuler l'une l'autre et le mouvement moyen total, D/n (où n est le nombre d'observations), serait petit (Diamond et Hankin 1985).

Les temps de liberté des crabes entre le marquage et la recapture ont été arrondis à 1 ou à 2 ans quoique le marquage ait été effectué en général à la fin de l'été ou à l'automne et que la recapture des crabes ait toujours eu lieu au printemps ou au début de l'été, en raison de la réglementation sur la saison de pêche.

TABLEAU 2. Résultats du test F de Watson–Williams et de U^2 de Watson sur les comparaisons d'orientations entre les trois différentes classes de taille des *C. opilio* mâles marqués à l'entrée de la baie des Chaleurs et dans la région des Îles de la Madeleine (dl : degrés de liberté).

Profondeur (m)	F	Prob. F	U^2	Prob. U^2	dl ν_1	dl ν_2
			Baie des Chaleurs			
< 94	0,09	> 0,25			1	50
94 à 105	1,57	> 0,1			2	174
> 105	69,81	< 0,001			2	211
			Îles de la Madeleine			
> 70[a]	1,81	> 0,1			2	256
70 à 81			0,048	> 0,5	36	15
> 81			0,030	> 0,5	149	52

[a]Données corrigées selon la pente.

Résultats

Sur 7 760 crabes mâles marqués dans les deux secteurs, 976 (12,6 %) marques ont été retrouvées avec des informations utilisables au cours des étés 1987 à 1989 (fig. 2). Ces crabes, recapturés 1 ou 2 ans après le marquage, ont parcouru en moyenne 9,6 km à l'entrée de la baie des Chaleurs et 13,7 km aux îles de la Madeleine (fig. 3). À l'entrée de la baie des Chaleurs, la distance maximale parcourue a été de 47,0 km et de 89,5 km aux îles de la Madeleine, toutes deux effectuées en moins de 1 an de liberté.

La maturité morphométrique a été déterminée selon l'équation discriminante de Conan et Comeau (1986), fondée sur la largeur de la carapace et la hauteur de la pince et établie pour les crabes de la même région. Selon le critère défini par ces auteurs, 94 % des individus recapturés étaient matures aux îles de la Madeleine et 97,4 % dans la baie des Chaleurs. On pourra donc considérer que les déplacements observés correspondent à ceux de la fraction mature de la population mâle.

Sur les 2 009 femelles marquées dans le secteur de la baie des Chaleurs, seulement six femelles ont été recapturées. La moyenne des parcours est de 3,9 km et les femelles ont toutes remonté vers la côte (profondeur de départ = 110,7 m, profondeur de recapture 92,5 m). Ces données ne sont pas incluses dans les analyses statistiques qui suivront.

Baie des Chaleurs

Dans la zone la moins profonde, < 94 m, les distances moyennes parcourues par les trois classes de taille, < 95, 95 à 114 et ⩾ 115 mm, sont de 8,7, 8,5 et 7,7 km respectivement. Ces moyennes ne sont toutefois pas significativement différentes (ANOVA : $F = 0{,}041$; $P_F = 0{,}96$; dl = 2 et 52). Dans la zone comprise entre 94 et 105 m, ces distances moyennes sont de 6,5, 8,4 km et 9,0 km respectivement et, ici encore, ne sont pas significativement différentes ($F = 0{,}655$; $P_F = 0{,}52$; dl = 2 et 174). Dans la zone la plus profonde (> 105 m), la distance parcourue augmente significativement avec la taille du crabe : LC < 95 mm, 3,6 km; 95 ⩽ LC < 105 mm, 8,8 km; LC ⩾ 105 mm, 13,2 km ($F = 9{,}258$; $P_F < 0{,}01$; dl = 2 et 211).

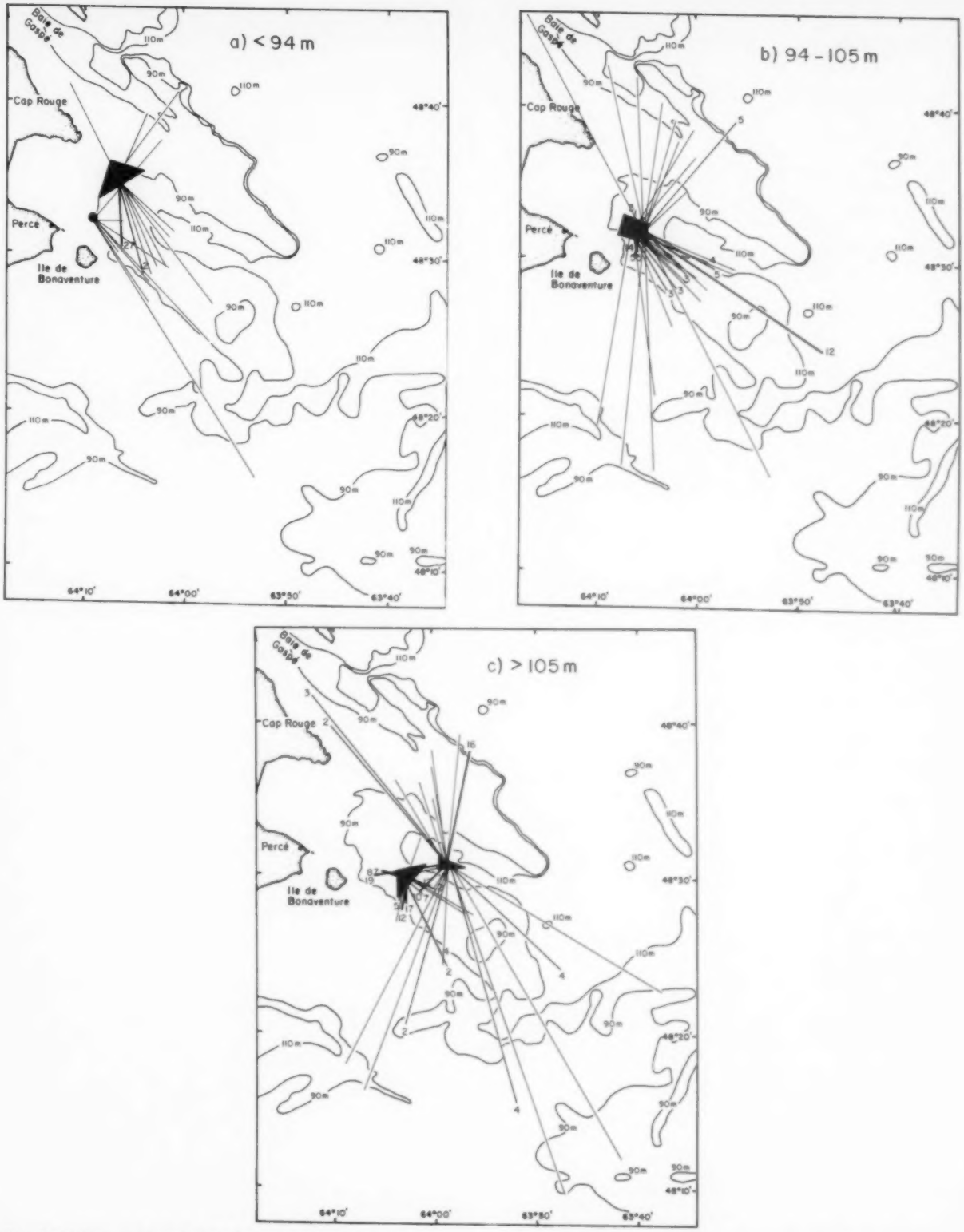

FIG. 4. Déplacements, après 1 ou 2 ans, des *C. opilo* mâles marqués dans la région de la baie des Chaleurs : a) zone peu profonde (< 94 m); b) zone intermédiaire (94 à 105 m); c) zone profonde (> 105 m). Chaque point représente une recapture ou plus, si indiqué.

TABLEAU 3. Résultats du test F de Watson–Williams sur les comparisons d'orientations entre les profondeurs pour les *C. opilio* mâles (95 à 115 mm LC) marqués à l'entrée de la baie des Chaleurs (n : nombre de recaptures; θ : angle moyen; dl : degrés de liberté).

Liberté	Profondeur (m)	n	θ (degrés)	F	Prob. F	dl ν_1	dl ν_2
1 an	< 94	27	180,9				
	94 à 105	107	10,1	51,65	< 0,001	2	241
	> 105	110	298,1				
2 ans	< 94	5	130,2				
	94 à 105	21	178,8	17,15	< 0,001	2	53
	> 105	30	270,4				

Il existe pour chaque classe de taille pour chacune des zones de profondeur une évidence statistique d'une direction moyenne prévilégiée par les crabes (tableau 1). Ces orientations ne diffèrent pas significativement entre les tailles pour les deux zones les moins profondes (tableau 2) et les crabes ont tendance en général à descendre (orientation entre 144 et 210°) vers les zones plus profondes (fig. 4a et 4b). Les crabes de la zone profonde, > 105 m, montrent des orientations variables selon la taille (tableau 2); les individus de 95 à 115 mm LC ont une tendance à la remontée vers la côte (fig. 4c).

Après 1 ou 2 ans de liberté, la distance parcourue par les crabes (95 à 115 mm LC) ne varie pas selon la zone de profondeur de marquage (1 an : $F = 0{,}039$, $P_F = 0{,}96$, dl = 2 et 241; 2 ans : $F = 0{,}743$, $P_F = 0{,}48$, dl = 2 et 53). Toutefois, la distance parcourue augmente significativement avec les années ($F = 4{,}318$; $P_F = 0{,}039$; dl = 1 et 298). Pour chaque année et pour chaque zone de profondeur, ces crabes possèdent une direction significative (pour toutes les directions, prob. $z <$ 0,01). Ces directions varient significativement avec la profondeur de départ, autant après 1 an que 2 ans de marquage (tableau 3). La tendance des crabes de la zone profonde à remonter est encore démontrée pour cette classe de taille.

Les vecteurs résultants moyens (D/n, Γ) pour les crabes dont LC = 95 à 115 mm indiquent effectivement une descente pour les deux zones les moins profondes et une remontée moins importante (D/n faible) pour les crabes marqués en zone profonde (fig. 5).

Îles de la Madeleine

La distance parcourue augmente significativement avec la taille du crabe, passant de 12,3 à 16,3 km pour LC < 95 et ≥ 115 mm respectivement, dans la zone la moins profonde, < 70 m (ANOVA : $F = 4{,}19$; $P_F = 0{,}016$; dl = 2 et 256). On observe la tendance inverse dans la zone la plus profonde, > 81 m, puisque la distance moyenne est de 23,8 km, pour LC < 95 mm, et de 13,7 km, pour LC ≥ 115 mm ($F = 3{,}40$; $P_F = 0{,}035$; dl = 2 et 213). La distance, variant de 13,9 à 8,8 km, ne diffère pas significativement avec la taille pour la zone intermédiaire, de 70 à 81 m ($F = 0{,}21$; $P_F = 0{,}81$; dl = 2 et 52).

Les distributions des orientations pour chaque classe de taille et pour chaque zone de profondeur démontrent une orientation moyenne significative (tableau 1) sauf pour les crabes de LC < 95 mm dans les deux zones les plus profondes. Peu importe leur taille, les crabes ont tous la même orientation dans la zone < 70 m (tableau 2; fig. 6a, 6b et 6c) et ils ont tous tendance à descendre la pente. Dans chacune des deux zones plus profondes, 70 à 81 et > 81 m (fig. 6d et 6e), les crabes de LC ≥ 95 mm ne montrent pas d'orientation différente selon leur classe de taille (tableau 2).

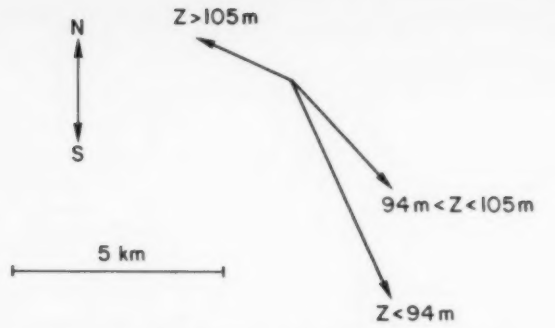

FIG. 5. Vecteurs résultants moyens directionnels (D/n : déplacement moyen total; Γ : angle, degrés) pour les *C. opilio* mâles (95 ≤ LC < 115 mm), marqués à l'entrée de la baie des Chaleurs (Z = profondeur de marquage).

Après 1 ou 2 ans de liberté, les crabes de 95 à 115 mm LC parcourent la même distance, entre 10,5 et 13,6 km pour 1 an et entre 11,5 et 18,2 km pour 2 ans, peu importe la profondeur de marquage ($F = 1{,}07$ et 0,97 respectivement; $P_F > 0{,}3$). Cette distance ne varie pas significativement avec les années de liberté ($F = 0{,}86$; $P_F = 0{,}35$; dl = 1 et 339). Ces crabes possèdent tous pour chaque zone de profondeur, une distribution circulaire ayant une direction moyenne significative (pour toutes les directions, prob. $z < 0{,}002$). Dans le secteur intermédiaire et le secteur profond, leurs orientations diffèrent significativement (tableau 4). Les crabes de la zone profonde ont un déplacement résultant $D/n = 5{,}0$ et d'orientation Γ = 35,9° (fig. 7) tandis que le vecteur résultant moyen des crabes de la zone intermédiaire indique que leur déplacement Γ = 355,0° et de moindre envergure ($D/n = 3{,}1$) : dans les deux cas, il s'agit d'un déplacement dans l'axe de la vallée. Dans la zone peu profonde, les déplacements se font dans le sens de la pente (Γ = 46,4°; $D/n = 4{,}6$).

La majorité des déplacements se situent principalement sur les abords immédiats de la vallée considérée. On constante néanmoins que six individus ont quitté ce secteur soit pour changer de vallée soit pour passer dans une autre dépression plus au nord.

Campagnes précédentes

Plusieurs individus ont été pêchés 3, 4 ou 5 ans après la campagne de marquage (tableau 5). Les distances parcourues paraissent élevées en comparaison des recaptures effectuées après 1 et 2 ans de liberté.

Discussion

Pour interpréter ces résultats ont doit considérer qu'il existe un biais important lié à ce type d'étude. Les données de retours des crabes proviennent des pêcheurs, et la distribution de l'effort de pêche influence considérablement la distribution des positions de recaptures. Des secteurs de pêche ont pu être favorisés par rapport à d'autres. Toutefois, ainsi que le remarquent Campbell et Stasko (1985), les pêcheurs commerciaux concentrent leur effort de pêche dans des zones où la ressource est la plus abondante, et déplacent leurs casiers vers des endroits

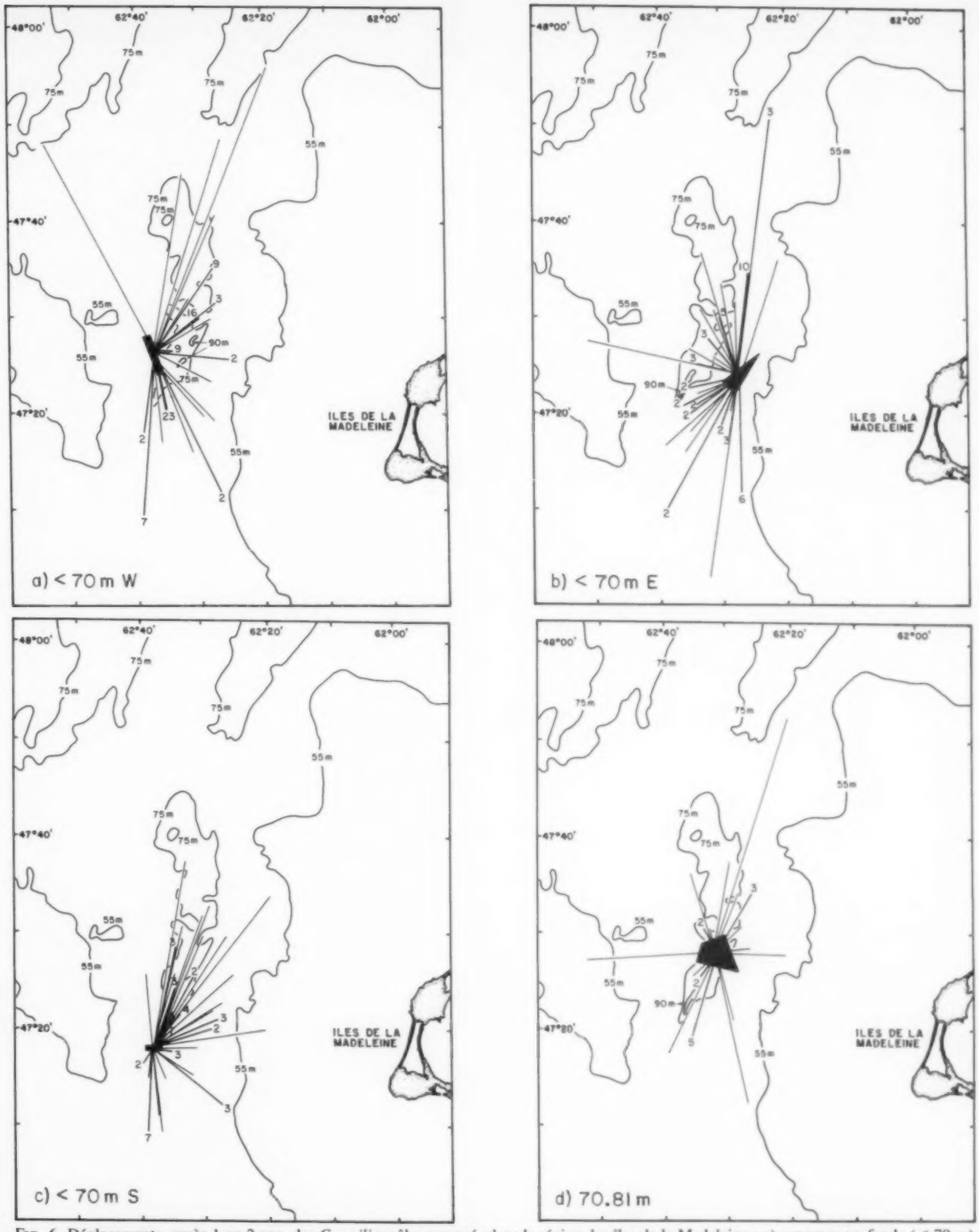

FIG. 6. Déplacements, après 1 ou 2 ans, des *C. opilio* mâles marqués dans la région des îles de la Madeleine : a) zone peu profonde (< 70 m) ouest (W), b) est (E) et c) sud (S); d) zone intermédiaire (70 à 81 m); e) zone profonde (> 81 m). Chaque point représente une recapture ou plus, si indiqué. (*Suite*)

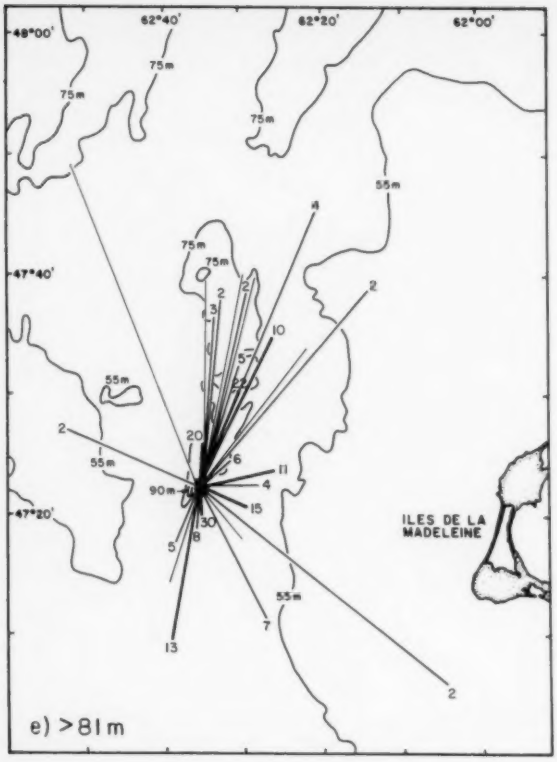

FIG. 6. (*Fin*)

TABLEAU 4. Angles moyens (θ) et résultats du test U^2 de Watson sur les comparaisons d'orientations entre deux zones de profondeur pour les *C. opilio* mâles (95 à 115 mm LC) marqués aux Îles de la Madeleine.

Profondeur (m)	*n*	θ (degrés)	U^2	Prob. U^2
< 70[a]	156	24,1		
70 à 81	36	56,8		
			0,459	< 0,001
< 81	149	58,9		

[a]Données corrigées selon la pente.

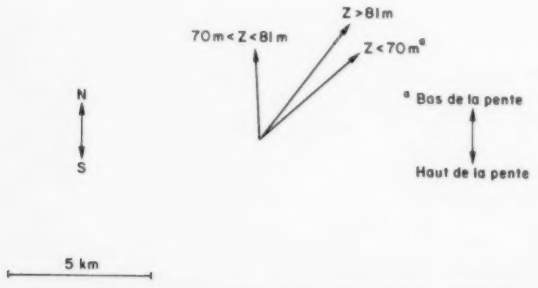

FIG. 7. Vecteurs résultats moyens directionnels (*D*/*n* : déplacement moyen total; Γ : angle, degrés) pour les *C. opilio* mâles (95 ≤ LC < 115 mm) marqués dans la région des îles de la Madeleine (*Z* = profondeur de marquage).

TABLEAU 5. Informations relatives aux recaptures des *C. opilio* mâles marqués à l'entrée de la baie des Chaleurs lors de campagnes précédentes ($\bar{d}$: distance moyenne parcourue).

Marquage	Recapture	Marques (*n*)	$\bar{d}$ (km)	d_{min} (km)	d_{max} (km)
9 octobre 1983	16 mai 1986	1	7,5		
	mai, juin 1987	2	18,6	9,3	27,9
	mai, juin 1988	10	19,4	6,3	57,5
5 à 8 août 1984	avril, juin 1987	14	16,4	5,5	29,7
	mai, juin 1988	5	40,2	38,2	45,3
26 septembre 1985	juin 1987	3	21,0	16,2	28,4
	28 mai 1989	1	0		

considérés comme profitables. Conséquemment, la distribution de l'effort de pêche réflèterait la localisation des concentrations de crabes mâles. Selon ces auteurs, le biais dû à l'effort de pêche, n'affecte pas les conclusions générales de ce type d'étude et seule la variance pourrait être affectée, moins que la direction moyenne.

De plus, il faut noter que la précision dans les positions de recapture variait beaucoup d'un pêcheur à l'autre. Les étiquettes à information de moins bonne qualité peuvent difficilement être traitées de façon différente puisque les pêcheurs ne nous renseignent pas sur la validité de leurs données. On constate néanmoins que, contrairement à d'autres études similaires (Watson 1970; Brêthes et Coulombe 1989), la position de libération est connue avec précision et que, dans l'ensemble la coopération des pêcheurs et des équipes scientifiques a été bonne, ce qui assure une certaine fiabilité aux résultats présentés.

Les résultats dépendant de la saison de pêche, il faut aussi tenir compte de la brièveté de cette saison (environ 10 sem) et qu'elle débute au printemps après le retrait des glaces. On ne dispose donc que d'une image de la position des *C. opilio* au cours d'un moment très bref de l'année.

Considérant ces divers biais, l'inférence à la population entière deviendrait risquée si la signification statistique d'un résultat provenait d'un artéfact (Diamond et Hankin 1985). Les déplacements eux-mêmes toutefois, sont des faits réels que l'on peut interpréter.

Dans cette étude, trois variables sont considérées : le secteur géographique (baie des Chaleurs, îles de la Madeleine), la largeur de la carapace (on a vu que les crabes considérés peuvent être considérés comme matures morphométriquement) et le secteur bathymétrique (zone peu profonde, intermédiaire et profonde). Des tendances générales se dessinent néanmoins. Tel qu'observé par Watson (1970) et Watson et Wells (1972), les mouvements demeurent de faibles amplitudes (d = 9,6 km dans le secteur de la baie des Chaleurs et 13,7 km aux îles de la Madeleine) quoique quelques déplacements importants aient été notés : 23 % des crabes des îles de la Madeleine ont parcouru au-delà de 20 km. L'amplitude des déplacements en fonction de la taille donne des résultats contradictoire. Il semble, en fait, que la largeur de carapace n'ait pas d'influence. Sur la côte nord du golfe du Saint-Laurent, Brêthes et Coulombe (1989) ont observés qu'une différence entre les classes de taille des mâles sur les vitesses de déplacement ne s'observait qu'immédiatement après la libération et qu'elle s'estompait après 1 an de liberté. Les délais impliqués ici expliquerait donc cette absence de différence, les plus petits individus ayant pu rejoindre les plus gros au cours de l'année.

Dans les deux secteurs géographiques, la taille n'influence pas non plus l'orientation prise par les crabes. Par contre une orientation privilégiée dépendra de la profondeur où les crabes ont été marqués. Ainsi, dans la région de la baie des Chaleurs, les crabes des niveaux bathymétriques supérieurs et intermédiaires ont tendance à descendre tandis que ceux du bas de la pente cherchent à remonter. Aux îles de la Madeleine, un schéma voisin est retrouvé : les crabes du haut de la pente sont descendu, ceux des zones intermédiaire et profonde sont généralement demeurés dans la coulée mais on observe également qu'une fraction non négligeable des individus profonds est remontée vers l'extérieur de la vallée.

Ces résultats confirment les hypothèses générales de Kon (1969, 1980), Coulombe (1984), Coulombe *et al.* (1985), Bouchard *et al.* (1986) et Brêthes et Coulombe (1989) selon lesquels les crabes mâles présent sur les niveaux supérieurs montrent un mouvement orienté vers les profondeurs.

Le mouvement des crabes de 95 à 115 mm LC des secteurs profonds vers les zones bathymétriques supérieures est similaire à celui observé à Bonne Bay (Terre-Neuve) (Taylor *et al.* 1985; Hooper 1986; Comeau *et al.* 1989). Ainsi, en considérant le fait que les crabes matures sont marqués à l'automne et recapturés au printemps, la tendance du mouvement observé peut être liée au déplacement lors de la reproduction printannière. Les six femelles retrouvées ont montré cette même tendance.

On doit sans doute considérer que les déplacements du crabe des neiges puissent être de deux types. Il y aurait un mouvement général descendant qui serait altéré par des mouvements saisonniers liés à la reproduction. C'est vraisemblablement au cours de mouvements ascendants saisonniers que les crabes localisés en profondeur pourraient être amenés à changer de sous-secteur géographique.

Les baies, coulées et autres obstacles majeurs très communs dans la région du golfe du St-Laurent, sont considérés affecter la direction et la distance du mouvement (Dufour 1988). Malgré la tendance générale des crabes marqués dans la région des îles de la Madeleine à demeurer dans la vallée de départ, une tendance à la dispersion amène quelques crabes à réussir à changer de coulée tout en effectuant des déplacements importants. Il n'est donc pas impossible que quelques communications existent entre les différents sous-stocks de pêche.

Remerciements

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Influence of Daily Variations of Light and Temperature on the Emergence Rhythm of Grayling Fry (*Thymallus thymallus*)

Agnès Bardonnet and Philippe Gaudin

Equipe d'Eco-éthologie, Laboratoire de Biologie Animale et Ecologie, 43, Boul. du 11 novembre 1918, F-69622 Villeurbanne Cedex, France

Bardonnet, A., and P. Gaudin. 1991. Influence of daily variations of light and temperature on the emergence rhythm of grayling fry (*Thymallus thymallus*). Can. J. Fish. Aquat. Sci. 48: 1176–1180.

The emergence rhythm of grayling fry (*Thymallus thymallus*) was studied in four aquaria fitted with traps under four different conditions of light and temperature. Both light and temperature induced an emergence rhythm characterized by a 24-h period. Under ambient light and constant temperature, emergence peaked at the end of the night before the onset of dawn. Under constant dim light and varying temperature, emergence peaked in early morning during the coldest hours. When both light and temperature fluctuated daily the emergence pattern was equivalent to the "sum" of these previous two regimes and was similar to that described in the field in a previous report. In the absence of light and temperature variations, no emergence rhythm was observed.

Une étude du rythme d'émergence d'alevins d'ombre commun (*Thymallus thymallus*) a été réalisée dans quatre aquariums équipés de pièges, sous quatre conditions distinctes de lumière et de température. La lumière et la température induisaient toutes deux un rythme d'émergence caractérisé par une période de 24 h. Avec les variations naturelles de lumière et une température constante, le pic d'émergence avait lieu en fin de nuit, avant l'aube. Avec une lumière constante atténuée et des variations circadiennes de température, le pic d'émergence avait lieu tôt le matin pendant les heures les plus froides. Lorsque la lumière et la température présentaient des variations journalières, l'image de l'émergence était comparable à la "somme" des deux rythmes précédents et était similaire au rythme décrit sur le terrain dans un précédent travail. En l'absence de variations de lumière et de température, aucun rythme d'émergence n'était observé.

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Emergence from the gravel bed by young salmonids is characterized by a diel rhythm. Most of the rhythms described are nocturnal and are presumed to represent an antipredator adaptation (Gustafson-Marjanen and Dowse 1983; Godin 1981). They are characterized by a peak during the first hours of the night (Gustafson-Marjanen and Dowse 1983; Brännäs 1987), and daily variations of light are usually considered responsible for such an emergence pattern. In a study on sockeye salmon fry (*Oncorhynchus nerka*), Bams (1969) found a strong correlation between variations of temperature and the level of activity of fry, the activity increasing with temperature. Other authors have studied the influence of temperature (Godin 1980; Brännäs 1987) but the daily variation of this factor was not considered and, in a general view, Smith (1985) indicated that the role of temperature in diel timing is limited.

Grayling fry (*Thymallus thymallus*) exhibit a diurnal emergence rhythm with a maximum early in the morning (Gaudin and Persat 1985; Bardonnet and Gaudin 1990a). Grayling larval biology is characterized by some peculiarities linked with its spawning behavior. No nests are dug and eggs are very close to the gravel surface (Fabricius and Gustafson 1955). This location could influence the expression of the daily rhythm, as daily variations of light and temperature are most detectable in the first centimetres of the gravel bed (Danielopol 1978; Bardonnet and Gaudin 1990b).

Comparing four incubation environments with different light and temperature regimes, we tested if daily variations of light alone accounted for the daily pattern of emergence in grayling or if either daily variations of temperature or a possible endogenous rhythm were involved in the expression of this rhythm .

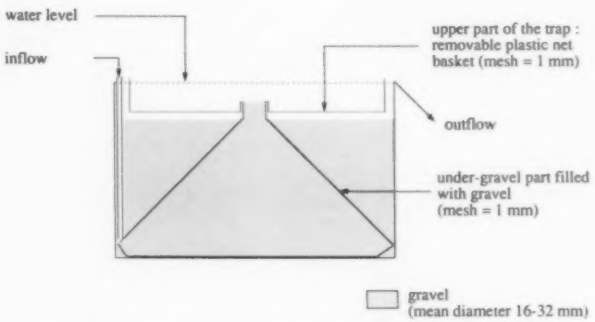

water level
inflow
upper part of the trap : removable plastic net basket (mesh = 1 mm)
outflow
under-gravel part filled with gravel (mesh = 1 mm)
gravel (mean diameter 16-32 mm)

FIG. 1. Profile of the experimental equipment used for trapping the emergent fry.

Material and Methods

Four aquaria (length = 40 cm, weight = 20 cm, height = 30 cm) with black walls were equipped with plastic net traps (mesh = 1 mm). Each trap consisted of an undergravel part shaped as a truncated cone filled and covered with gravel (diameter 16–25 mm) and a free-water part fitted to it, consisting of a removable plastic net basket (Fig. 1). Two 100-L tanks were equipped with sensitive thermoregulators, one kept at a constant temperature of 12 ± 0.1°C and the other at a temperature

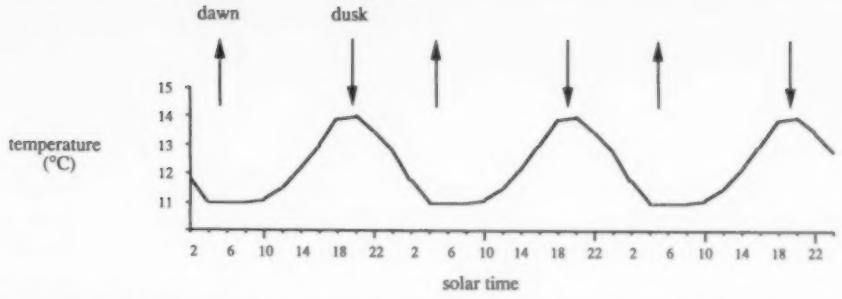


FIG. 2. Characteristics of the temperature rhythm during experiments A1 and A3. Light intensity cycled daily in experiment A1; dawn and dusk are indicated.

TABLE 1. Survival at the time of emergence under the four experimental conditions.

	A1	A2	A3	A4
Number of fry	187	185	221	181
% survival	62	62	74	60

that varied between 11 and 14°C, with a daily average of about 12°c (Fig. 2). These daily variations were chosen in accordance with field measurements and provide suitable incubation temperatures for grayling (Humpesch 1985). Each tank provided a constant flow of water (15 L/h) for each of two aquaria. For each temperature treatment, one aquarium was kept under constant dim-light conditions (0.1 lx) and the other under natural light/dark variations (from 0.05 to 4000 lx). Hence, four experimental situations were created: aquarium A1, daily variations of light and temperature; aquarium A2, daily variations of light, temperature kept constant; aquarium A3, daily variations of temperature, light kept constant; aquarium A4, light and temperature kept constant.

On 23 March 1988, gametes of two males (37 and 41 cm) and three females (38–41 cm), caught in the Suran river (Ain, France), were mixed and 300 eggs placed inside each undergravel trap 7 h after fertilization. Traps were checked daily and from the first catch sampled every 3 h (from 01:00 to 22:00, solar time). Two additional checks were carried out at dawn (when light increased above 0.2 lx, at about 03:30 ± 15 min) and dusk (when light decreased below 1 lx, at about 19:30 ± 15 min) in order to separate diurnal from nocturnal catches.

The results were analysed using the chi-square periodogram technique of Sokolove and Bushell (1978). This method is derived from the Enright formulation of periodogram analysis (1965, cited in Sokolove & Bushell 1978). By taking χ^2_{P-1} (where P represents the number of tallies for the tested period) as the null distribution, they tried to establish the significance of a Q_p periodogram peak (Q_p represents the ratio between the variance of P means of K items drawn from a population of N values and the total variance of these values normally distributed). By simulating the behavior of Q_p, they showed that the integral-valued chi-square periodogram could distinguish circadian periodic series from random series at a level of significance of about 0.01.

Results

Emergence took place over 10 d from 15 to 24 April after 276–320 degree-days of incubation and survival was similar in the four tests (Table 1). Both the length of the emergence period and the daily rhythm differed among treatments.

Length of the Emergence Period

The length of the period necessary to trap 90% of the emerging fry (by elimination of an equal portion of 5% at both ends) varied between treatments. It was longer in A2 (6 d) than in A1 (5.1 d) and A4 (4.9 d) and in A3 (4.1 d) (A2 > A1, A4 > A3). In A1 and A2 the fish emerged later (starting 16 April) than in A3 and A4 (starting 15 April, with high amplitude already on 16 April) (Fig. 3).

Emergence Rhythm

When light and temperature were constant (A4), there was no daily periodicity (Fig. 4). The few peaks of activity produced contained no rhythm as judged by periodogram analysis (Fig. 5) and have been considered as random events. When light and temperature varied (A1; Fig. 4), the general pattern of activity was similar to the natural pattern of emergence described by Bardonnet and Gaudin (1990a). Periodogram analysis showed a 24-h rhythm, but this was not statistically significant at the 1% level (Fig. 5), possibly because the emergence peak did vary by one or two time steps among days. However, maximum emergence was observed consistently at the end of the night and early morning (for solar hour intervals 01:00–04:00, 04:00–07:00, 07:00–10:00). In the field, light and water temperature variations are linked, while in our experiments this link was not so clear, the temperature variations being regularly fixed. This phenomenon might have been one of the factors responsible for the differences in the daily position of the emergence maxima observed in A1.

When only light varied (A2), emergence peaked strongly at the end of the night (Fig. 4) and the 24-h periodicity was significant ($p < 0.01$; Fig. 5). When only temperature varied (A3), emergence started strongly during the time interval 04:00–07:00, which corresponded with the thermal minimum, decreased during the warming period (10:00–19:30), and became almost nil when the temperature reached its maximum (19:30). It started again only when the temperature again reached its minimum (Fig. 4). The period of 24 h was significant ($p < 0.01$; Fig. 5).

Discussion

Variations in the timing of the emergence period between the four treatments were observed. Tanks A3 and A4 were in a

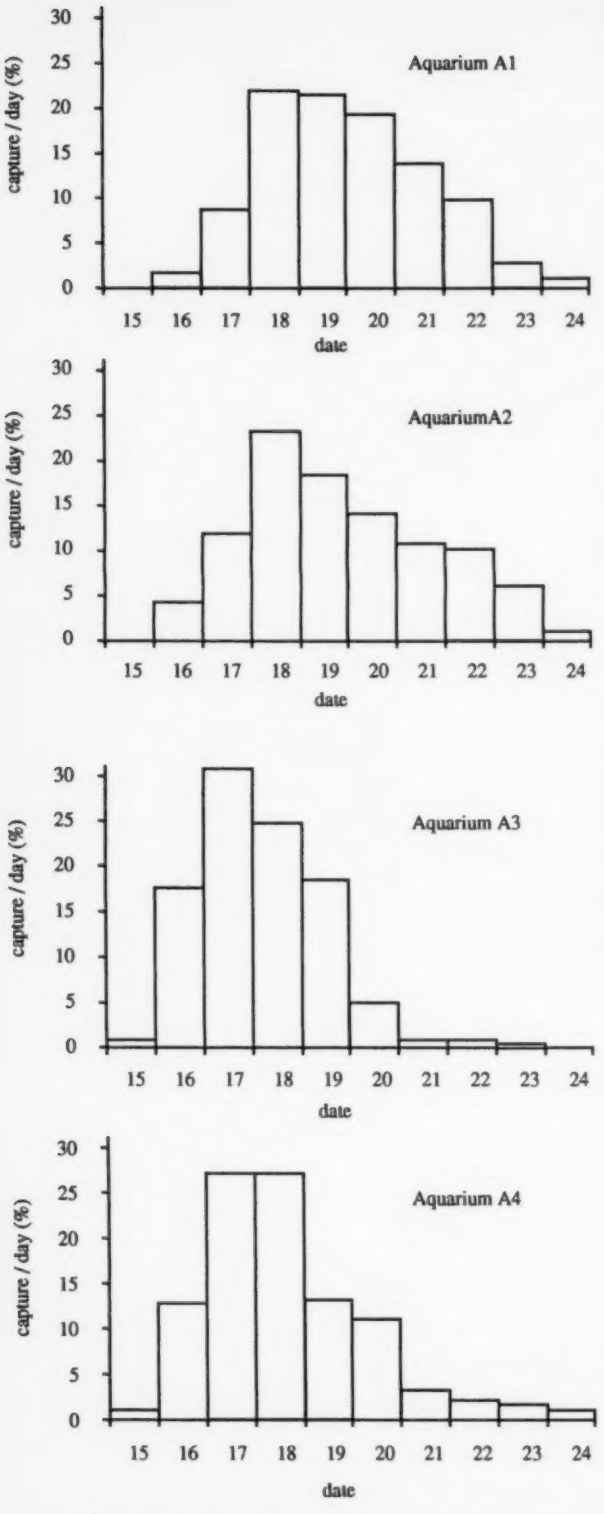


FIG. 3. Pattern of emergence for the four experimental conditions, 15–24 April.

dark room and about 0.3° warmer than tanks A1 and A2 during daylight (because of the sun on the wall of the dark room). For the whole period of development this represented a difference of 5–9 degree-days. This could explain the lag of 1 d in the start of emergence in tanks A1 and A2, but it is also possible that light had an inhibitory effect delaying emergence. However, the warming inside the dark room was not important enough to explain the differences in the length of the period necessary to complete 90% of emergence. The variations observed in the length of the emergence period may be related to the action of the two synchronizers (light and temperature). If the period of possible emergence within each 24 h (assumed to be the period when biotic and abiotic factors determine emergence) was shortened by the presence of one or both synchronizers, this could increase the number of days necessary for complete emergence.

The emergence rhythm in grayling clearly responded to daily variations of both temperature and light. When temperature varied alone, maximum emergence occurred during the coldest hours. Animal activity usually increases with temperature (Palmer 1976), and Bams (1969) demonstrated it for Pacific salmon fry emergence. This did not occur in grayling, but there may be an optimal temperature for activity nearer 11°C than 14°C in this species. However, the characteristics of the experimental daily rhythm of temperature (maxima and minima) were chosen from field measurements in order to be close to natural conditions, and in the field at the time of emergence, water temperature below 11°C is rarely observed. Therefore it is possible that for grayling fry, emergence temperature act as a "Zeitgeber." When light varied alone the emergence peak was well marked, closely associated with the sharp and rapid change in the level of illumination at dawn and dusk. However, the peak did not occur at the beginning of daylight nor at the beginning of night. A lag existed between the time when alevins got the signal and the time when they emerged. In the absence of variations in light and temperature, the daily emergence pattern was arhythmic, but this absence of evidence for an endogenous rhythm (Bünning 1964) is not proof of its absence. Brady (1982) distinguished in the behavioral animal's rhythms two qualitatively different kinds of behavior which are rhythmic: the "gated," once-in-a-lifetime programmes (birth, hatching, moulting), measurable on groups and the "daily-repeated," ongoing behavior that is measurable in individual animals. Emergence occurs only once in the alevin's life and if it is a gating mechanism the rhythmicity can be pointed out only at a population level. If each individual was expressing its own endogenous rhythm, in the absence of temperature or light cues, these rhythms were not synchronised at the population level. Most of experiments on the emergence rhythm have been performed at this level, and if some results confirmed (Brännas 1987) or infirmed (Bams 1969) an endogenous component, none of them closed the debate. However, our results do not allow us to exclude that emergence is a behavior induced by a daily-repeated rhythm corresponding to the daily activity of the fry. At an individual level, the results of Perez (1986) have shown that under dim-light conditions, four embryos (out of five that were tested) exhibited an organized activity with a period varying from 10 to 30 h, depending on each individual. However, the test of autocorrelation was significant for only two of them.

When both light and temperature varied, one did not override the other, but both affected the emergence pattern. At an individual level, these results could indicate that the time of emergence was determined by an endogenous event, specific to each

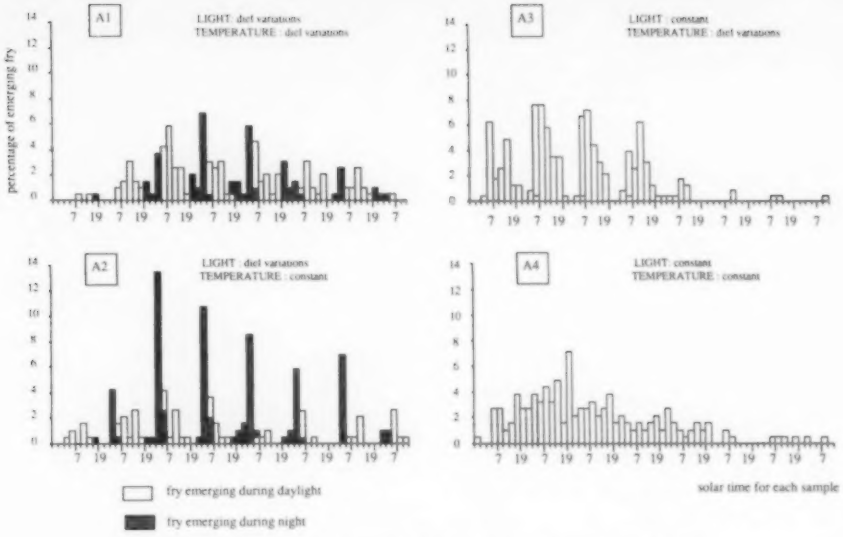
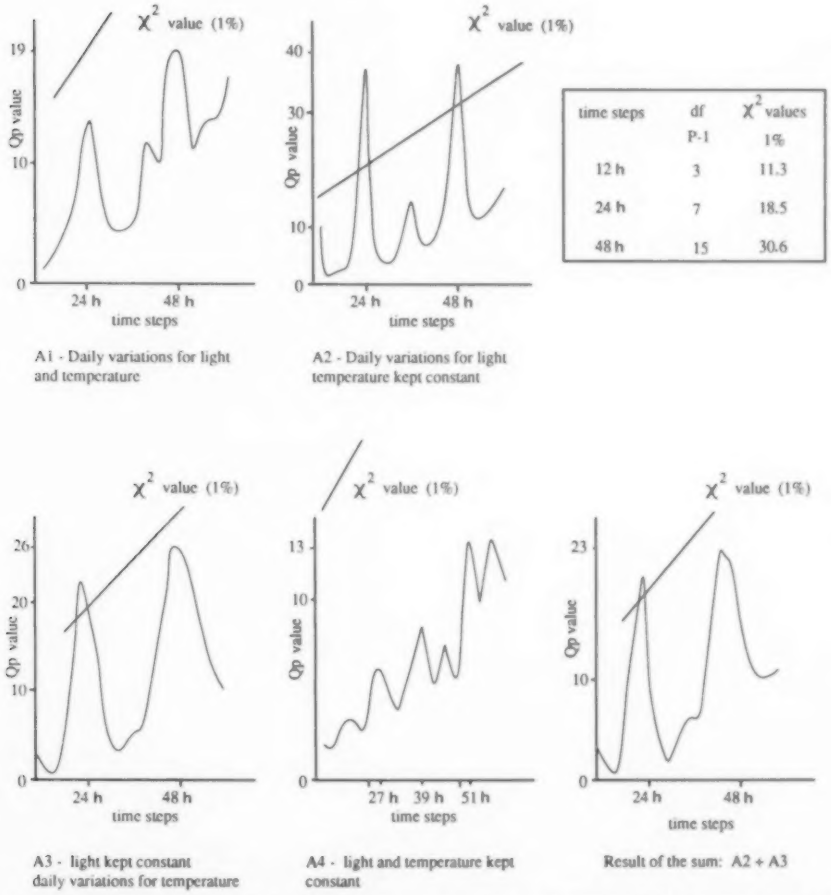


FIG. 4. Daily emergence pattern for the four experimental conditions.


χ² value (1%)
Qp value
24 h
48 h
time steps
A1 - Daily variations for light and temperature
A2 - Daily variations for light temperature kept constant
A3 - light kept constant daily variations for temperature
A4 - light and temperature kept constant
27 h 39 h 51 h
Result of the sum: A2 + A3

time steps	df	χ^2 values
	P-1	1%
12 h	3	11.3
24 h	7	18.5
48 h	15	30.6

FIG. 5. Pictorial representation of the periodogram analysis. The *x*-axis represents the period *p* (time step 3 h) and the *y*-axis the values of Q_p at χ^2_{p-1} df. The critical χ^2 values for the various degrees of freedom are tabulated. In this analysis, days with less than five individuals caught per 24 h were eliminated.

individual, which determined a time interval of possible emergence. During this interval, the actual time of emergence depended on when light and temperature conditions were optimal for emergence. Indeed, it is improbable that individual differences in the levels of sensitivity to light and/or temperature could induce a response to a light synchroniser for some individuals and a temperature synchroniser for other individuals.

The emergence pattern obtained under ambient light and temperature conditions was very close to the natural one (Bardonnet and Gaudin 1990a). Variations in light and temperature decreased rapidly with depth (Danielopol 1978; Crisp 1990). Thus, the depth of the nest could have great importance for the perception of the daily variations of the environmental conditions. The relatively shallow depth of grayling nests would imply greater exposure to daily variations in light and temperature than in the deeper nests of other salmonids. Indeed, the "Zeitgeber" for the emergence of grayling could be specific to them and thus explain the differences between the rather diurnal pattern of emergence in grayling and the nocturnal one of other salmonids. It is now necessary to test the role of daily variations of light and temperature on the daily pattern of emergence in *Salmo* species.

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Variations in Year-Class Strength of Different Perch (*Perca fluviatilis*) Populations in the Baltic Sea with Special Reference to Temperature and Pollution

Paula Böhling, Richard Hudd, and Hannu Lehtonen

Finnish Game and Fisheries Research Institute, Fisheries Division, P.O. Box 202, SF-00151 Helsinki, Finland

and Peter Karås, Erik Neuman, and Gunnar Thoresson

Swedish Environmental Protection Agency, Coastal Laboratory, P.O. Box 584, S-740 71 Öregrund, Sweden

Böhling, P., R. Hudd, H. Lehtonen, P. Karås, E. Neuman, and G. Thoresson. 1991. Variations in year-class strength of different perch (*Perca fluviatilis*) populations in the Baltic Sea with special reference to temperature and pollution. Can. J. Fish. Aquat. Sci. 48: 1181–1187.

Variations in the year-class strength of perch (*Perca fluviatilis*) were analyzed among 23 populations in Baltic coastal areas distributed between 57 and 66°N using the age distribution in catches. In spite of large differences in abiotic and biotic factors, the variations in year-class strength were similar in 14 of these areas. The similarities could be attributed to large-scale weather variations influencing water temperature. Year-class strength was shown to be correlated with an index based on temperature and day length during the whole first year of life. In two of the nine deviating populations, perch nursery areas are situated in small nearshore freshwaters, where fluctuations in the water level may be more important for recruitment than temperature variations. The other deviating areas were exposed to environmental disturbances, i.e. acidification, oxygen deficits, pulp mill effluents, and thermal discharge, which obviously affected recruitment in a decisive way.

On a analysé les variations de l'effectif des classes d'âge de 23 populations de perches *Perca fluviatilis* de secteurs de la côte de la mer Baltique situés entre les 57 et 66° de latitude N en se basant sur la distribution des âges dans les prises. En dépit d'importantes différences de nature abiotique et biotique, les variations d'effectif étaient semblables dans 14 des secteurs. Les ressemblances observées pourraient s'expliquer par l'influence des grandes variations météorologiques sur la température de l'eau. On a montré que l'effectif des classes d'âge est en corrélation avec un indice combinant la température et la longueur du jour durant la première année de la vie des poissons. Les aires de reproduction de deux des neuf populations déviantes se trouvaient dans de petites zones d'eau douce littorales, milieu où les fluctuations du niveau de l'eau influent peut-être davantage que les variations de température sur le recrutement. Les autres zones déviantes étaient exposées à des perturbations environnementales telles qu'acidification, manque d'oxygène, rejet d'effluents d'usines de pâtes et papiers ou d'effluents thermique, facteurs qui, de toute évidence, ont influé de manière déterminante sur le recrutement.

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Along the Swedish and Finnish coasts of the Baltic Sea, salinity is low enough for maintenance of freshwater fish populations. These coasts are generally shallow and rich in inlets and islands, offering sheltered and warm habitats suitable for fish species with comparatively high temperature preferences. Cyprinids and percids are common, and the perch (*Perca fluviatilis*) is generally one of the dominant species (Neuman 1977, 1982; Ojaveer et al. 1981). It is also an important object for angling and commercial fishing; the Finnish catches in the Baltic were estimated to 3000 tons in 1988 (Finnish Game and Fisheries Research Institute, pers. comm.).

The regulation of year-class strength is a question of major interest when interpreting monitoring data and for predictions of stock sizes. In the Baltic, variations in the strength of perch year-classes are large and have been found to be positively correlated with summer water temperature during the first year of life (Neuman 1976), a frequent observation in percids (Koonce et al. 1977). According to Karås (1987), temperature affects first year survival of perch in the Baltic indirectly through its positive effects on consumption and growth; a large size was considered to reduce mortality due to predation and winter starvation.

The main objective of this paper is to test the hypothesis presented by, for example, Koonce et al. (1977) that large-scale weather variations influence perch survival causing similar patterns in the variation of year-class strength over large geographic areas containing widely different habitats. Such comparisons were possible in the Baltic Sea, as perch age sampling programmes exist in a number of areas extending from 57 to almost 66°N, covering different climates and day length conditions. The areas also represent a broad spectrum of topographic and hydrographic conditions, different productivity levels, general fish community and ecosystem structures, as well as fishing pressures. Perch populations with recruitment areas in the sea, in estuaries, and in nearshore freshwaters connected with the sea are represented. Areas with different degrees and types of environmental disturbances are included. The different investigation areas contain separate stocks, as the perch

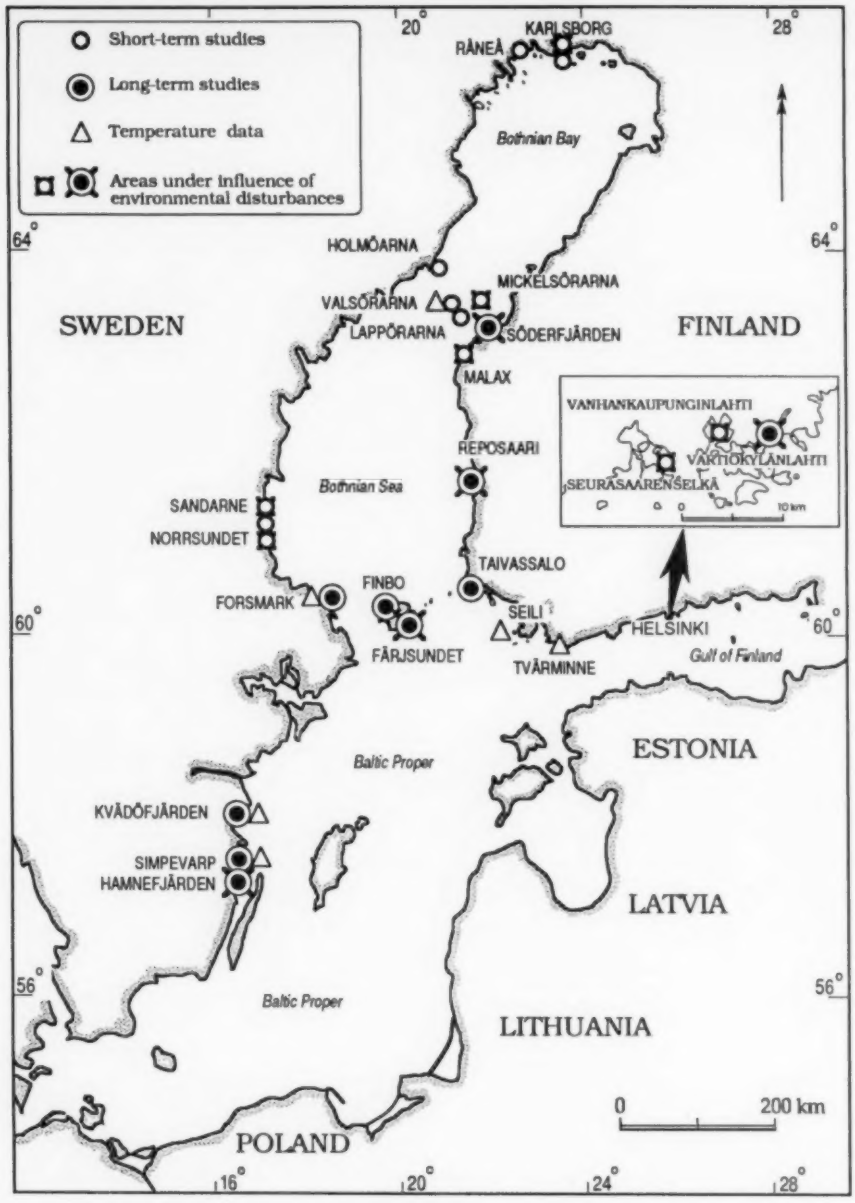

FIG. 1. Sampling sites.

in the Baltic is relatively stationary (Böhling and Lehtonen 1984; Hudd et al. 1984).

Study Area

The Baltic Sea is the largest brackish water basin in the world. In the southern part of the study area at Simpevarp, western Baltic proper (Fig. 1), the salinity of the surface water is 7‰ compared with only 1–3‰ in northern Bothnian Bay. The tide is insignificant. On average, the nearshore waters are ice covered for 2 mo in the Baltic proper and for more than 6 mo in Bothnian Bay. The interyear variations are small in Bothnian Bay but large in the Baltic proper, where ice-free winters occur. In the open sea the maximum summer surface temperature is usually 13–15°C. Considerably higher temperatures, 20–25°C, may be reached in perch recruitment areas, e.g. sheltered archipelagoes, bays, estuaries, rivers, and small lakes connected with the sea. Temperature variations between years are large, however, and in many areas the 20°C level is only occasionally reached. There is no clear south–north gradient in the maximum temperatures in the coastal areas, but the length of the perch fry growth season, i.e. the period warmer than 10°C (Karås 1987), generally decreases northwards. Information concerning environmental characteristics in the different study sites is summarized in Table 1.

Biotic factors which may influence perch recruitment vary widely in different parts of the Baltic. Primary and secondary production generally decrease northwards (Sandström and Sörlin 1979; Melvasalo et al. 1981; Elmgren 1984). Predation and competition may also change with latitude; e.g. coldwater species such as the coregonids are more common and warmwater fishes such as cyprinids and perch less common in Bothnian Bay than in the Baltic proper (Ojaveer et al. 1981). The investigated perch stocks were also exposed to highly differing fishing pressures. Due to differences in the size and quality of perch recruitment areas and in fishing intensity, a variety of parental stock sizes may be expected.

Material and Methods

Perch were collected yearly from 10 coastal areas on a multiyear basis (1978–87, or part thereof) (Table 1; Fig. 1). Supplementary samplings were made in 13 additional areas in 1985 and 1986 (Table 1; Fig. 1). The perch sampled were sexed and aged. Scales (Segerstråle 1933) and operculae (Le Cren 1947) were used in ageing.

Samplings were performed with gill nets, wire traps, trap nets, pound nets, and fyke nets (Table 1). The gill nets were either survey nets or links. Survey nets are 35 m long and 3.0 m high and are composed of five 7-m-long parts with different mesh sizes (17, 21, 25, 33, and 50 mm bar length). A link consists of a combination of nine 30-m-long and 1.8-m-high nets (21, 25, 2 × 30, 2 × 33, 38, 50, and 60 mm bar length). Wire traps, width 1.2–1.4 m, are made of iron wire with a mesh size of 25 mm. In the river Malax, perch were caught using trap nets (mesh size 13 mm) during spawning migrations from the Baltic. Pound nets had a large 13-m-long bag with a 50- to 100-m-long and 6.5-m-high leader and a mesh size of 12–14 mm. The fyke net used had a 60-m-long leader with a mesh size of 24 mm. The mesh size at the end of the gear was 11 mm.

The long-term sampling was used to calculate indices for relative year-class strengths according to the method of Svårdson (1961), adjusted by Neuman (1974). Indices were calculated for the year-classes 1973–81 using the yearly age distributions. Calculations were restricted to ages 4–7, i.e. to fishes that were old enough to be recruited to all sampling gears and to ages that were reasonably well represented in the catches. The calculations were made on data from both males and females, as the differences in age distributions between sexes usually are not significant in the studied age groups (Böhling 1988). Year-class strengths in an area were estimated stepwise beginning with the calculation of the percentage age distributions (4–7 yr) in the yearly samplings. Thereafter the mean percentage age distribution for the whole period was established. In the next step the different year-classes in different years were expressed as percentages of this mean distribution. Thus, if 4-yr-olds in 1979 (year-class 1975) comprised 20% of the 4- to 7-yr-old perch compared with 40% in the mean distribution in the actual area, they reached 50% of the average this year. If the corresponding value was 70% in 1980 (5 yr), 60% in 1981 (6 yr), and 60% in 1982 (7 yr), the index of the strength of the year-class 1975 would be 60%. Means based on less than 3 yr were omitted.

TABLE 1. Environmental and sampling characteristics of the investigated areas.

	Environmental				Sampling				
Area	Geographic character	Salinity (‰)	Ice cover (d)	Environmental impact	Sampling method	Year	Month	Sample size	Sex
Karlsborg	Estuary	0–2	190	Pulp mill	Survey net	1985	8	179	♂♀
reference[a]	Estuary	2	190		Survey net	1985	8	508	♂♀
Råneå	Estuary	0–2	190		Wire trap	1986	5	193	♂
Holmöarna	Archipelago	4	150		Wire trap	1985	5	179	♂
Valsörarna	Archipelago	4	150		Wire trap	1985	6	92	♂♀
Lappörarna	Archipelago	4	160		Wire trap	1985	6	261	♂♀
Mickelsörarna	Archipelago	4	150	Acidification	Wire trap	1985	6	183	♂♀
Söderfjärden	Estuary	0–3	160	Acidification	Wire trap	1981–87	5–6	919	♂♀
Malax	Estuary	0–4	140	Acidification	Trap net	1985	5–6	400	♂♀
Reposaari	Archipelago	5	130	Pulp mill, eutrophication	Wire trap	1981–87	4–6	1545	♂♀
Sandarne	Archipelago	5	110	Pulp mill	Survey net	1985	8	298	♂♀
Norrsundet	Archipelago	5	110	Pulp mill	Survey net	1985	8	208	♂♀
reference[a]	Archipelago	5	110		Survey net	1985	8	338	♂♀
Forsmark	Archipelago	5	100		Fyke net	1978–83	5–6	1421	♀
					Survey net	1984–87	8	1374	♀
Finbo	Archipelago	6	80		Survey net	1978–81, 83–87	8	1925	♀
Färjsundet	Archipelago	6	100	Eutrophication	Pound net	1978–79, 1981, 1983–86	6[c]	2129	♂♀
					Wire trap	1987	6	454	♂♀
Taivassalo	Archipelago	6	120		Pound net	1978–87	5–6	2599	♂♀
Vartiokylänlahti	Archipelago	5	120	Eutrophication	Wire trap	1978, 1980–87	5–6	3000	♂♀
Vanhankaupunginlahti	Archipelago	4	120	Eutrophication	Wire trap	1986	5–6	146	♂♀
Seurasaarenselkä	Archipelago	5	120	Eutrophication	Wire trap	1986	5	191	♂♀
Kvädöfjärden	Archipelago	7	70		Net link	1978–87	5–6	1071	♀
Simpevarp	Archipelago	7	60		Net link	1978–87	5–6	729	♀
Hamnfjärden[b]	Inlet (0.1 km^2)	7	0	Heated water	Net link	1978–87	5–6	787	♀

[a]Endpoints in pollution gradient studies considered as references.
[b]Situated within the Simpevarp area; heating started 1971.
[c]Months 5–6 before 1983.

For the 1-yr samplings, year-class strengths cannot be normalized. Thus, just age distributions, expressed as a percentage of the total catch, were calculated. Data from 1985 were chosen for fast-growing stocks and 1986 data for slow-growing ones in order to reduce effects of differences in growth rate and, consequently, in age of recruitment. This classification was mainly based on growth data presented by Böhling (1988).

For analyzing the effect of temperature on variations in year-class strength, water temperature data for the years 1973–81 were used (Fig. 1). In all areas except Kvädöfjärden and Seili (representing Taivassalo), temperatures were measured at locations too exposed to represent perch recruitment areas. In comparisons concerning interyear temperature patterns in different parts of the Baltic, locations too exposed can, however, be accepted and temperatures from six areas were used. Temperatures were measured daily at Valsörarna, Seili, and Tvärminne, three times per week at Forsmark, and weekly at Kvädöfjärden. At Simpevarp, temperatures were measured twice a month during 1973–76 and daily during 1977–81. When temperatures were measured less frequently than daily, they were interpolated to obtain daily values.

To analyze the influence of temperature on recruitment in the study areas Kvädöfjärden and Taivassalo, correlations between year-class strength and temperature during the first year of life were calculated using two different approaches. The first implies a linear relationship between temperature and recruitment during the period of maximum weight gain (July–August) and the second predominantly an exponential relationship based on the whole first year. In the second approach, weights after the first year of life were used as temperature index. The weight growth was calculated from a growth model (Karås 1987) considering the effects of temperature on spawning, embryonic development, and larval growth and the influence of both temperature and, as perch is a visual feeder, day length on fry growth. Fry growth was simulated in a bioenergetics model assuming that food is not limiting growth in the studied populations. During periods with ice cover the consumption was assumed to stop due to low light conditions.

Results

The interrelation between the 10 areas in relative year-class strength was analyzed (Kendall's coefficient of concordance (W), Conover 1971) but no significant correlation could be demonstrated ($W = 0.29$). However, if the five areas influenced by environmental disturbances were excluded (Table 1), the correlation between the remaining five areas (Fig. 2A) was statistically significant ($W = 0.55$, $P < 0.01$). The general pattern in year-class strength thus established was compared with the observations in each of the five influenced areas by pairwise linear correlation analysis. The highest correlations were found for the Reposaari and Vartiokylänlahti materials (0.62 and 0.67, respectively) (Fig. 2B); the highest of the other coefficients was 0.32 (Fig. 2C). The correlation was statistically significant for Vartiokylänlahti ($P < 0.05$) but not for Reposaari due to the low number of year-class estimates.

In the correlated areas, a strong perch year-class appeared in 1980 and generally relatively weak ones in 1976–79 and 1981 (Fig. 2A). The 1979–82 year-class strength patterns in the areas with single-year sampling were generally similar to this pattern (Fig. 3). However, Valsörarna and Lappörarna deviated as well as some of the polluted areas (Fig. 1; Table 1). When comparing all the 23 perch populations studied, including the reference areas to the pulp mills at Karlsborg and Norrsundet, it is evident that variations were similar in 14.

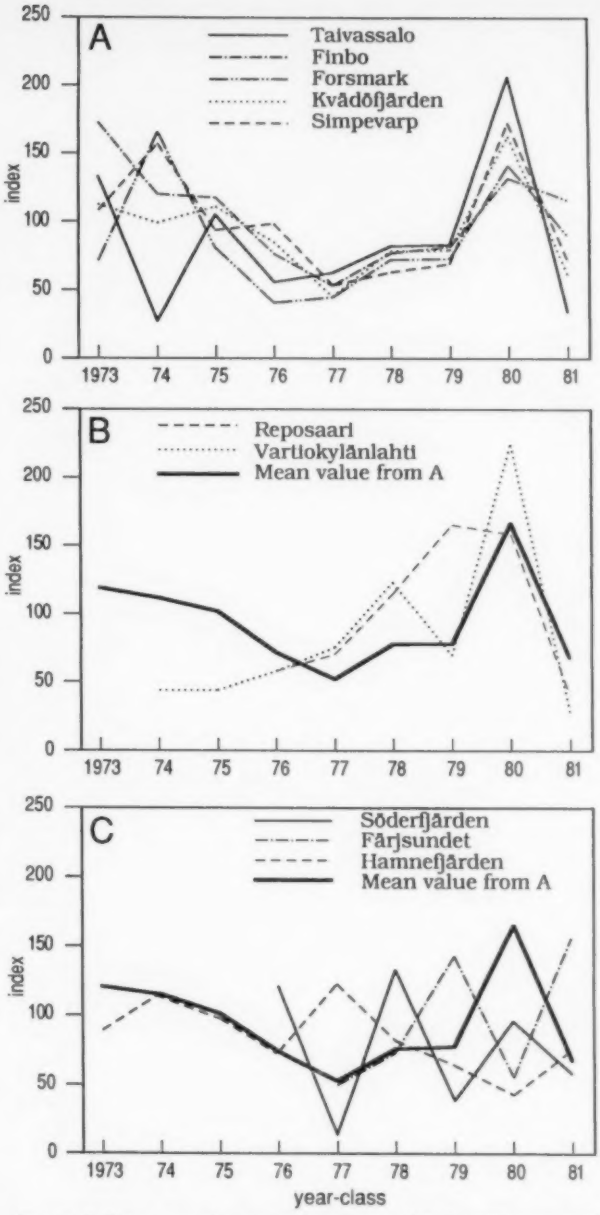

FIG. 2. Relative year-class strength (mean = 100) for different perch populations calculated from age distributions within the period 1978–87 (A) Dominating year-class pattern; (B) areas with environmental disturbances (broken and dotted lines) that demonstrate patterns similar to the dominating one (bold line) calculated as the mean of the areas in Fig. 2A; (C) areas with environmental disturbances (thin, broken–dotted, and broken lines) demonstrating patterns deviating from the dominating one (bold line).

Pairwise linear correlation analysis employed on the long-term monitoring material for both relative year-class strengths and temperature data demonstrated that all areas were significantly positively correlated with at least one of the others

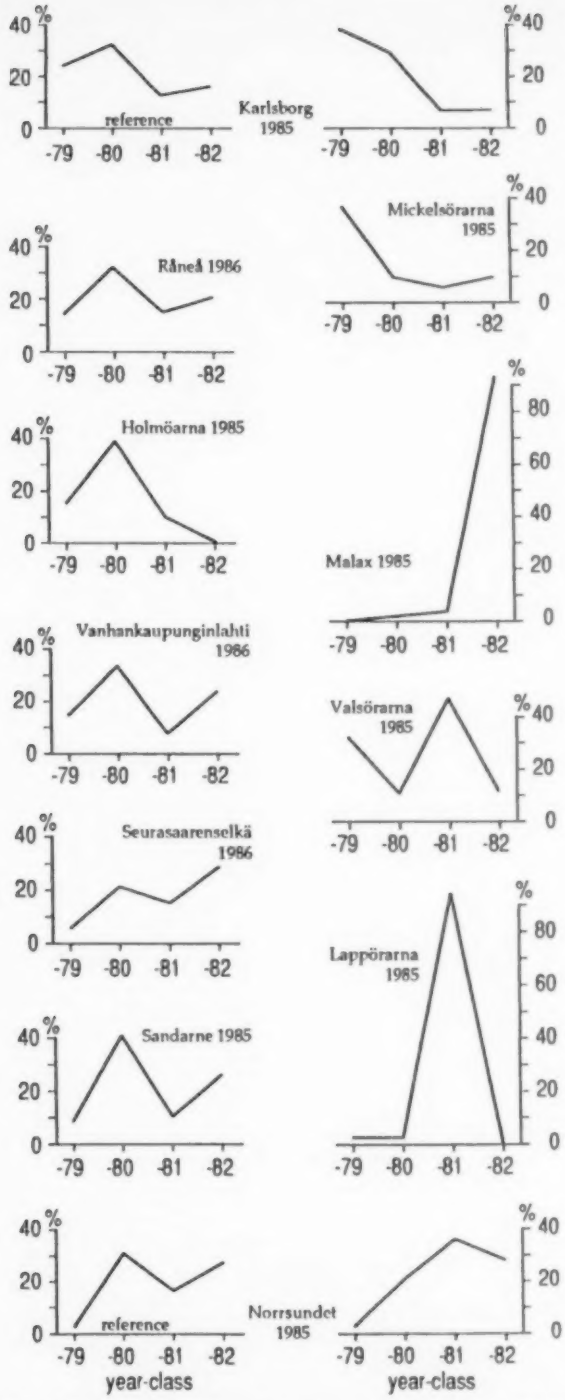

FIG. 3. Percentage of different year-classes in 1-yr samples. The left panels represent the dominating year-class pattern and the right ones the deviating patterns.

(Fig. 4). Areas that vary in a similar manner with respect to water temperature and year-class strength from year to year are generally situated on the same side of the Baltic, the correlations between Kvädöfjärden and Taivassalo/Seili being the only exceptions.

In the study of temperature effects on recruitment, correlation coefficients were calculated between relative year-class strengths and two different yearly temperature indices for Kvädöfjärden and Taivassalo. The correlations for the whole first year of life were higher (0.71 and 0.78, respectively) compared with the shorter period of July–August (0.57 and 0.68). All the values except 0.57 are statistically significant ($P < 0.05$). A graphic illustration of the concurrent variation between the relative year-class strength and the index representing the whole first year of life demonstrates good predictive capacity of this model (Fig. 5).

Discussion

For the Baltic, it was demonstrated that the variations in the year-class strength of perch were similar in populations spread over a large area. Thus, the year-classes 1973–81 were correlated between seven areas, and there were obvious similarities between seven additional populations sampled in a single year, representing the year-classes 1979–82. As shown earlier for perch in the Baltic (Neuman 1976), there was a positive correlation between year-class strength and summer temperature. The highest degree of explanation was achieved, however, when year-classes were related to a growth model (Karås 1987) based on temperature and day length during the whole first year of life.

The correlations between areas as regards year-class strengths as well as temperatures were strongest between areas situated on the same side of the Baltic (Fig. 4); the only exception was between the most sheltered areas (Taivassalo/Seili and Kvädöfjärden). Large-scale weather variations causing similarities in the interyear fluctuations in summer temperature and warming in spring provide the most probable reason for this general agreement. It is difficult to find any alternative physical or biological factor acting similarly over such a large area. The most probable explanation of the fact that the strongest association was established within the different sides of the Baltic is that a certain wind direction influences water temperatures differently along the west and east coasts; e.g. the predominating southwesterlies tend to replace surface water with colder water on the western but not on the eastern side. Such upwellings sometimes cause dramatic drops in temperature which not only reduce the growth rate, but may also cause mortality of the larvae (Karås 1987). The positive correlation between Taivassalo and Kvädöfjärden is probably due to the fact that these recruitment areas are situated in large and dense archipelagoes impeding upwellings.

Fishing pressure and biotic factors such as food availability, parent stock size, and interspecific interactions vary widely between the sampling areas. The results indicate that such factors did not affect the interyear variations in recruitment in a decisive way. For a Baltic perch population, Karås (1987) discussed the influence of food availability and predation on recruitment. He considered predation pressure to be important and negatively correlated with fry growth rate. The year-class strength thus is affected by temperature through its positive effect on first-year growth. Food availability was regarded as less important for year-class regulation, as the consumption of perch fry was not limited by access to food in temperatures below 20°C, a temperature level that is surpassed only for shorter periods in Baltic waters.

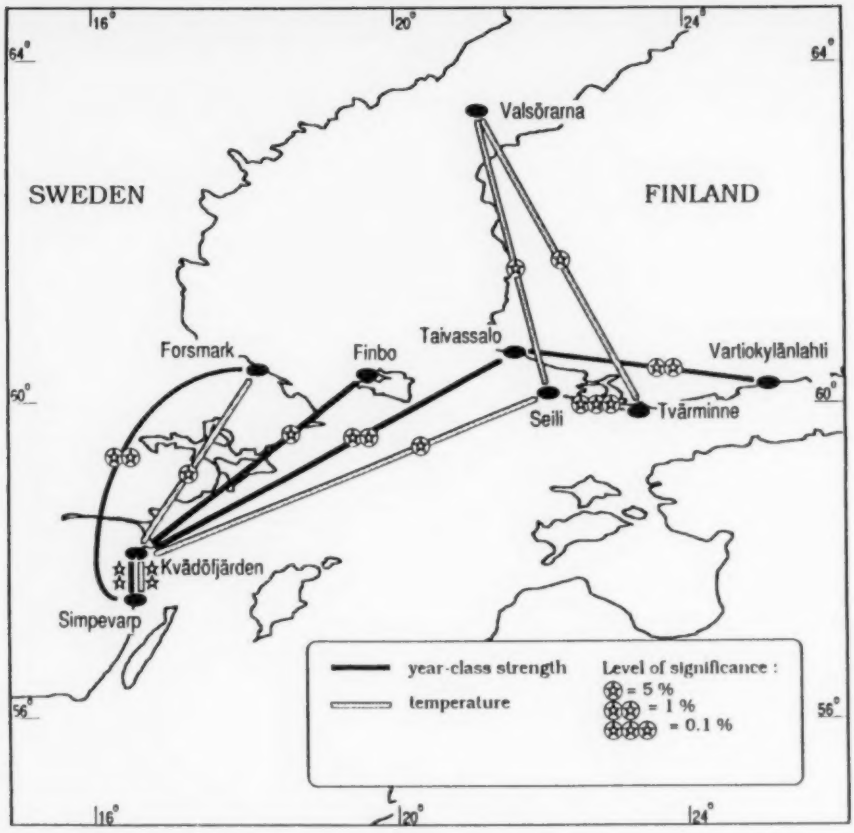

FIG. 4. Correlations between relative year-class strength and temperature conditions. All sampling areas were compared in a statistical correlation analysis. Significant positive correlations are shown in the figure. Finbo, Vartiokylänlahti, and Taivassalo were excluded from the temperature comparison and Valsörarna, Seili, and Tvärminne from the year-class comparisons due to lack of data.

In the archipelagoes separating Bothnian Bay and the Bothnian Sea, all sampling areas except Holmöarna deviate from the dominating year-class pattern. Valsörarna and Lappörarna (Fig. 3) are groups of islands where the perch largely spawn in small and very shallow nearshore freshwaters. Human activities are insignificant and the most important regulator of reproductive success probably is the water level at the spawning sites, which fluctuates widely between years due to variations in precipitation. Thus, exceptional rainfalls in June and August 1981 (Finnish Meteorological Institute, Wolffintie 36, SF-65200, Vaasa 20, Finland, unpubl. data) may explain the strong year-class of 1981. Dependence of recruitment on lake water level has also been demonstrated for yellow perch (*Perca flavescens*) by, for example, Henderson (1985). Söderfjärden, Mickelsörarna, and Malax (Fig. 2C and 3) are influenced by runoff from rivers damaged by dredgings causing acid shocks and leakages of toxic metals, seriously reducing recruitment in several fish species (Hudd et al. 1984).

Not only acidification but also other environmental disturbances caused deviations from the natural year-class pattern, as in two areas affected by pulp mills (Karlsborg and Norrsundet, Fig. 3). At Norrsundet, extensive studies in fact have demonstrated that recruitment was severely impaired by toxic effluents (Karås et al. 1991). One deviating area (Färjsundet, Fig. 2C) was influenced by agricultural runoff and organic loading from a food processing industry, occasionally leading to oxygen deficit (C. Storå, Ålands landskapsstyrelse, Fiskeribyrån, Box 60, SF-22101 Mariehamn, Finland, pers. comm.). Aberrant patterns also occurred in the area receiving cooling water from a nuclear power plant (Hamnefjärden, Fig. 2C), increasing the water temperature 5–10° above ambient. There the generally positive effects of a warm summer on fry survival are reduced or even reversed when the optimal temperature is surpassed.

Five of the studied populations influenced by pollution followed the natural year-class pattern. Two of them were affected by pulp mill effluents (Sandarne and Reposaari) and four by eutrophication (Reposaari, Vanhakaupunginlahti, Seurasaarenselkä, and Vartiokylänlahti). Obviously these disturbances did not vary particularly between years or are not severe enough to overrun the natural determinants of year-class strengths in a significant way; e.g. oxygen deficits do not occur in these recruitment areas.

To conclude, the results of this investigation support the hypothesis that large-scale weather variations, mainly mediated via water temperature, synchronize variations in year-class strengths of perch over a large area with highly variable habitats. The big differences between areas as regards abiotic environment, community structure and productivity, etc., must lead

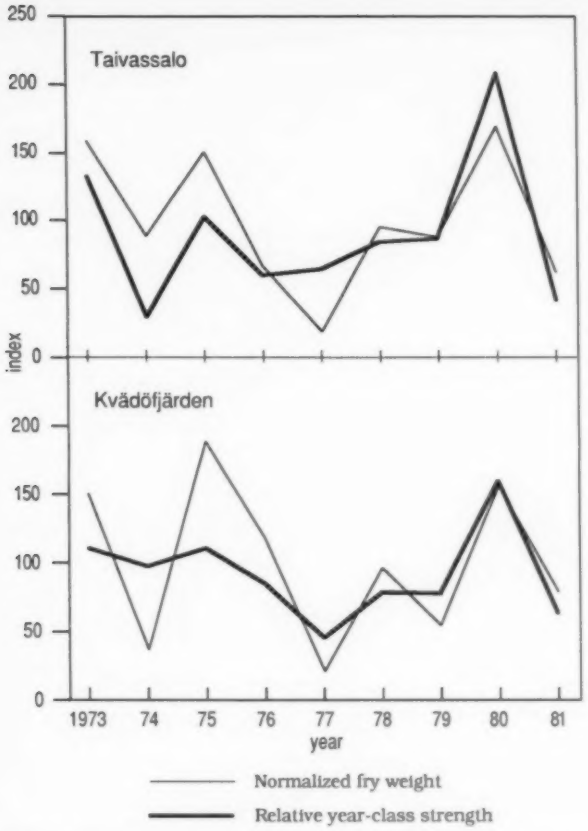

FIG. 5. Comparison between relative year-class strength and fry weight after the first year of life calculated according to Karås (1987). Both variables are normalized around 100.

to basic differences in general abundance of the species, but obviously have only minor influence on the interyear variations in year-class strength. Environmental disturbances, however, may affect recruitment so profoundly that the influence of temperature is masked or overrided.

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Paternal Association of Increased Susceptibility to Viral Haemorrhagic Septicaemia (VHS) in Rainbow Trout (*Oncorhynchus mykiss*)

P. Kaastrup[1,2] and V. Hørlyck

Danish Trout Culture Research Station, Brøns Mølevej 7, DK-6780 Skærbæk, Denmark

N. J. Olesen, N. Lorenzen,[2] and P. E. Vestergaard Jørgensen

National Veterinary Laboratory, Hangøvej 2, DK-8200 Århus N., Denmark

and P. Berg

Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen, Denmark

Kaastrup, P., V. Hørlyck, N. J. Olesen, N. Lorenzen, P. E. Vestergaard Jørgensen, and P. Berg. 1991. Paternal association of increased susceptibility to viral haemorrhagic septicaemia (VHS) in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1188–1192.

Farming of rainbow trout (*Oncorhynchus mykiss*) in Europe is hampered by unacceptably heavy losses due to the severe infectious disease viral haemorrhagic septicaemia (VHS). Strain-dependent variation of VHS resistance exists. A long-term breeding programme to increase VHS resistance in rainbow trout has been started in Denmark. This programme will be based on experimental VHS challenge of the parental fish ($n = 84$) as well as their normal and gynogenetic offspring (16 fullsib F1 groups). We found a paternal influence on the average VHS resistance of the offspring; partial regression coefficients for sire-offspring were estimated at 0.30 ± 0.09 and for dam-offspring at -0.10 ± 0.12.

En Europe, l'élevage de la truite arc-en-ciel (*Oncorhynchus mykiss*) progresse peu en raison de pertes excessives dues à une maladie infectieuse, la septicémie hémorragique virale (SHV). La résistance à la SHV varie selon la souche. Au Danemark, on a lancé un programme de reproduction à long terme en vue d'augmenter la résistance de la truite arc-en-ciel à la SHV. Ce programme est fondé sur la provocation expérimentale à la SHV des poissons reproducteurs ($n = 84$) ainsi que de leur progéniture normale et gynogénétique (16 groupes de pleins-frère, pleines-soeurs de F1). Nous avons constaté qu'il s'exerce une influence paternelle sur la résistance moyenne à la SHV de la progéniture; nous avons estimé le coefficient de régression partielle : $0,30 \pm 0,09$ pour les descendants des mâles et $-0,10 \pm 0,12$ pour les descendants des femelles.

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Viral haemorrhagic septicaemia (VHS) is a serious disease of farmed rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) caused by Egtved virus (Jensen 1965). Strain differences in the resistance to the causative virus may exist in rainbow trout (P. E. Vestergaard Jørgensen, unpubl. results), and nearly complete resistance has been described for coho salmon (*Oncorhynchus kisutch*) and triploid rainbow trout female × coho salmon male hybrids (Dorson and Chevassus 1985). However, there is no knowledge of the number and kind of genes involved in the resistance/susceptibility patterns.

This report describes recently obtained experimental evidence of a purely paternal influence on VHS susceptibility in F1 generations of rainbow trout. These F1 generation fish were produced with the long-term goals of improving the genetic resistance in rainbow trout broodstocks to Egtved virus by using a forced selective breeding program and of investigating the possible role of the putatively highly polymorphic major histocompatibility complex (MHC) (Klein 1986) in the resistance patterns of trout to VHS.

Methods

Ninety-three sexually mature rainbow trout (1.5–2.0 kg, 63 females, 30 males), with a record of freedom from VHS as well as the vertically transmissible diseases infectious pancreatic necrosis (IPN) and bacterial kidney disease (BKD), and originating from a strain of rainbow trout known to be relatively resistant to experimental VHS, were transferred to our research station where they were individually marked.

The local trout stock of the research station itself is free from BKD but latently infected with IPN virus. All the marked trout were involved in random matings. Normal F1 generation groups (63) and corresponding gynogenetic F1 generation groups (60) resulting from these matings were propagated at the research station according to the methods previously described (Kaastrup and Hørlyck 1987).

[1]Author to whom correspondence should be addressed.
[2]Affiliated Institute for Experimental Immunology, University of Copenhagen, as Ph.D. students.

After the matings had been performed, all parent trout were transferred to the National Veterinary Laboratory (NVL) and, after acclimation, the fish (now reduced to 61 females and 23 males) were challenged with Egtved virus by bath infection in city tap water at 10°C as previously described (Jørgensen 1982). Two separate challenge experiments (A: 32 females, 16 males; B: 29 females, 7 males) were performed for reasons of capacity limitations. The virus used was a mixture of seven wild strains serologically related to the reference strain "F-one" of Egtved virus. The virus concentration in the water was 10^6 plaque-forming units (PFU)/mL. All trout that died during the first week of the experiment ($n = 25$) were examined individually in a virus isolation test involving inoculation of organ homogenate into BF-2 cell cultures.

When the F1 generation groups reached the average size of 5 cm, two random samples (2 × 25 fullsibs) from each of 18 selected F1 groups were transferred to NVL, placed in 10-L aquaria (25 sibs in each), and exposed to bath infection with Egtved virus at a concentration of 10^5 PFU/mL water. The 18 F1 groups selected for VHS challenge were from mating combinations which represented the outermost extremes with regard to parental VHS resistance. The VHS challenge of the F1 groups were also temporally separated in two experiments, C and D, due to limitations of aquaria capacity. A random pool of F1 generations and a random sample of 5-cm fish from farm M which delivered the broodstock were included in the challenge experiments as negative (− VHS virus) and positive (+ VHS virus) control fish groups, respectively. These fish had been reared in farm M at a water temperature and diet similar to that used for the F1 groups. The number of dead fish was recorded daily for 3 wk in all challenge groups. Samples of fish dying at the onset and at the peak of the mortality were examined for the presence of characteristic VHS haemorrhages in swimbladder, muscle, and brain. Seven fish from the pooled F1 group, serving as negative controls in the challenge experiment, were submitted to virological examination individually by inoculation of organ homogenate into BF-2 cell cultures.

Statistics

Median survival time of the different group combinations was calculated and the data submitted to the median test according to the principles outlined by Siegel (1956). As a measure of resistance to VHS infection the reciprocal values of number of days to death were used, smaller figures indicating higher resistance. This transformation stabilized the variance and normalized the distribution. Resistance data were then submitted to analysis of variance (ANOVA).

The model used to investigate parental resistance was

$$(1)\quad Y_{ijk} = \mu + K_i + S_j + KS_{ij} + e_{ijk}$$

where Y_{ijk} = resistance (reciprocal days to death) of the kth fish of sex j in challenge group i, μ = mean, K_i = effect of challenge group (i = A, B), S_j = effect of sex (j = male, female), KS_{ij} = interaction between sex and challenge group, and e_{ijk} = the residual assumed to be identically and independently distributed $(0, \sigma^2)$ ($k = 1,\ldots,84$).

The model used to investigate the relationship between parental and offspring VHS resistance was

$$(2)\quad Y_{ijkl} = \mu + \beta_1 \cdot x_{1i} + \beta_2 \cdot x_{2j} + G_k + e_{ijkl}$$

where Y_{ijkl} = average resistance in aquarium 1 of fullsib group from sire i and dam j in challenge group k (average calculated as the average of individual reciprocal days to death), μ = mean, x_{1i} (x_{2j}) = resistance of sire (dam) ($i = 1,\ldots,12$, $j = 1,\ldots,15$), β_1 (β_2) = partial regression of offspring resistance on sires (dams) resistance, G_k = effect of challenge group (k = C, D), and e_{ijkl} = residuals ($l = 1,\ldots,30$) (assumed to be independently and identically distributed $(0, \sigma^2/N_1)$; N_1 is the number of fullsibs in aquarium 1). Estimates of β_1 and β_2 were obtained by the method of weighted least squares.

Results

The experimental VHS challenge of the parent trout led to death with typical symptoms of VHS of 94% of the fish during the first 15 d after challenge (Fig. 1) and revealed a lower median survival for the male fish (sires, m = 6 d) than for the females (dams, m = 8 d). Virus was readily isolated from all the 25 examined trout. Model 1 showed no significant interactions between the sex of the parents and the challenge group on resistance ($F_{1,80} = 0.02$, $P > 0.50$). Model 1 without the interaction term (KS_{ij}) showed a significant difference between sexes ($F_{1,81} = 51.59$, $P < 0.001$) but not between challenge groups ($F_{1,81} = 0.01$, $P > 0.50$). This was not surprising, since mature rainbow trout males under farming conditions often show higher mortality due to infectious diseases than do mature females. The parents were assigned a VHS "survival rank" (H for relatively high survival, M for relatively medium survival, and L for relatively low survival) according to their individual survival time in days (dams: H = survival time >9 d, L = survival time <7 d; sires: H = survival time >7 d, L = survival time <5 d). The necessary selections for continued propagation and VHS challenge of the most interesting F1 groups from the previously random mating combinations were based on this ranking of the parents.

Challenge of the offspring resulted in heavy mortality starting 3 d after the infection. The symptoms observed were typical of VHS. Virus was not isolated from any of the examined negative F1 control fish. The latter finding makes it unlikely that an IPN infection, possibly initiated during the stay of the fish at the research station, could have influenced the mortality in the VHS challenge experiments. No significant difference was found between the two sets (C and D) of challenge experiments with the F1 groups as indicated by the median survival of the control fish (Table 1). The challenge of the F1 offspring belonging to H × H crosses (high-rank female fish × high-rank male fish) showed that these had a significantly higher combined median survival time than those belonging to L × L crosses ($P < 0.001$, median test). This indicates a considerable genetic influence from the parents on the median survival time of the offspring (Table 1).

Figure 2 shows a plot of the average VHS resistance of the offspring versus the VHS resistance of the parents and suggests a predominant role of the male parents. In order to obtain a more precise estimate of the association of sex with this apparent heritability of resistance to VHS, model 2 (see Methods) was used. The distribution of resistance was slightly positively skewed for males and negatively skewed for females. The ANOVA of model 2 and the estimated partial regression coefficients are shown in Table 2. In the case of autosomally inherited resistance to VHS, the expected heritability is two times the regression of offspring resistance on the resistance of one parent. The analysis shows that the resistance of the sires has a significant influence on the resistance of the offspring ($F_{1,26}$

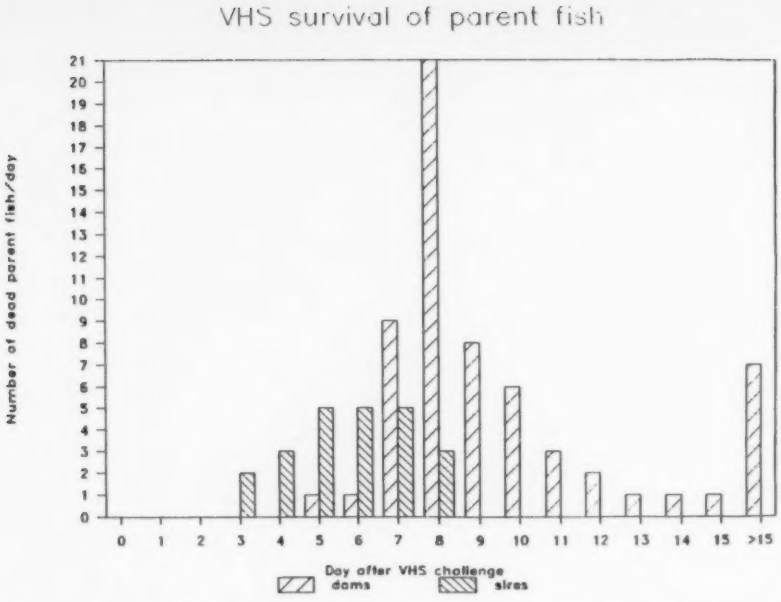
VHS survival of parent fish
Number of dead parent fish/day
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 >15
Day after VHS challenge
dams
sires

FIG. 1. Daily mortality for each sex of the rainbow trout parents (n = 84) after experimental VHS infection.

TABLE 1. Median survival of F1 generation groups challenged with VHS and parental VHS survival.

F1 group (dam × sire)	Parental VHS cross	Experimental group	Median survival of F1 from two aquaria tests (d)	Parental survival (d)	
				Dam	Sire
62 × 57	H × H	D	15	16	8
43 × 42	H × H	C	7	10	8
71 × 70	L × H	D	7	5	8
38 × 28	L × H	C	7	7	7
8 × 7	H × M	C	8	15	6
10 × 7	H × M	C	15	13	6
32 × 26	H × L	C	8	100	5
30 × 26	L × L	C	7	7	5
56 × 58	L × L	D	10	6	5
47 × 46	H × L	C	5	100	4
55 × 54	H × L	C	6	11	4
49 × 48	L × L	C	6	7	4
59 × 60	H × L	D	6	16	3
84 × 88	H × L	D	5	11	3
83 × 88	L × L	D	7	7	3
50 × 52	H × ?	C	12	50	?
Gynogenetics					
84 × (88)[a]	H[a]	D	9	11	(3)[a]
83 × (88)[a]	L[a]	D	12	7	(3)[a]
Control groups					
Farm M, VHS challenge		C	6		
Farm M, VHS challenge		D	5		
Farm M, no VHS challenge		D	>15		
F1 pool, no challenge		C	>15		
F1 pool, no challenge		D	>15		

[a]UV-irradiated sperm.

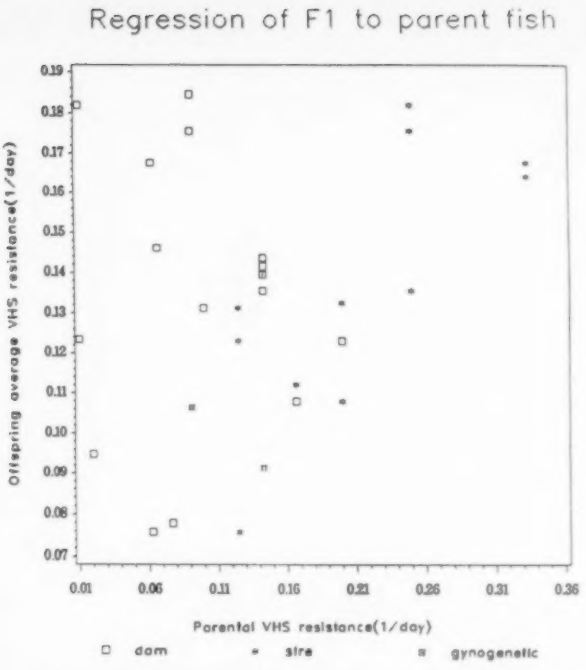

FIG. 2. Average VHS resistance of offspring plotted with parental VHS resistance (see Table 1).

TABLE 2. ANOVA of the regression of offspring resistance on parent resistance using model 2. ** $P < 0.01$; NS = nonsignificant ($P > 0.3$).

Source	df	Type 3 SS	MS	F
Sire	1	0.33314	0.33314	11.31**
Dam	1	0.02189	0.02189	0.74 NS
Challenge group	1	0.00231	0.00231	0.08 NS
Error	26	0.76572		

Regression	Estimate	SE
Sire-offspring β	0.30	0.09
Dam-offpsring β	−0.10	0.12

= 11.31, $P < 0.01$), but the resistance of the dams has no significant influence ($F_{1,26} = 0.74$, $P > 0.30$).

Unfortunately, few gynogenetic groups produced enough individuals for experimental VHS challenge. However, we compared the combined median survival times of the gynogenetic F1 groups from one high (H) and one low (L) VHS survival ranked female with those of the corresponding normal crosses (H × L or L × L) which involved the same (L) male fish (Table 1). As expected, the elimination of the genome from the sires with low VHS survival times resulted in an increased median survival time in both gynogenetic groups ($P < 0.001$, median test). It appeared, however, that gynogenetic offspring of the low-resistance female as well as those of the high-resistance female showed at least as long median survival time as the combined median survival time of the expectedly superior crosses (H × H).

Discussion

The present selection experiment was performed with males and females of the same commercial broodstock, which was most unlikely to be genetically uniform. Genetic variation within the broodstock is also implied by skin transplantation and by serological studies of rainbow trout blood cells by the use of rainbow trout alloantisera raised against lymphocytes of individuals belonging to the rainbow trout strain used in this study. These data have shown the strain to be polymorphic (P. Kaastrup, unpubl. data), essentially as previously described for common carp (*Cyprinus carpio*) Kaastrup et al. 1989).

The main, and unexpected, finding in the present experiment is that the resistance to a standard challenge with VHS virus, as judged by survival time after infection, was positively and significantly correlated with the resistance of the male parent. Resistance seemed independent of that of the female parent. However, this observation was based on progeny that average 5 cm in body length.

We also found, from VHS virus challenge of the actual parents after spawning, that all males died within 8 d. The females had a median survival time of 8 d and included several long-term survivors. The very high virus concentration used for challenge was chosen in order to obtain the strongest possible selection for resistance.

Unfortunately, we do not know whether young fish with incipient gonad differentiation also display a sex difference in resistance to VHS infection. If, in fact, they do, random fluctuations from a 1:1 sex ratio in our samples of challenged offspring may represent an error that makes the interpretation difficult. New experiments in progress aim at elucidating whether the sex of immature, normal trout influences the resistance to VHS. In addition, experiments with offspring from sex-reversed females, or gynogenetic offspring, sex-reversed by suitable, early hormone treatment, might elucidate whether the inferior average resistance of males is due to the male phenotype or to some other property determined by the Y chromosome.

Observations on rainbow trout chromosome morphology (Thorgaard 1977) have shown a distinct polymorphism of different male trout Y chromosomes. Some of these appear to show visible deletions whereas others seem to be nearly indistinguishable from the X chromosome. A possible and simple explanation for our observations might be that both the Y chromosome and the X chromosome carry a gene, or a cluster of genes, that is important for VHS resistance and located in the region of the frequently observed deletions on the Y chromosome. Females would then always be equipped with a double gene dose of their putative resistance genes whereas males, depending on the extent of their Y chromosome deletion, would be equipped with a single dose only of the deleted genes. An implication of this hypothesis is that only the male offspring of a low-resistance father will inherit his low resistance.

If paternal inheritance plays indeed the major role in the hereditary resistance to VHS in rainbow trout, autosomal genes, including MHC genes, may play a minor role only. However, polymorphic loci of importance for VHS susceptibility could be located on autosomes, although somehow regulated by genes of the Y chromosome. Theoretically this might be an alternative to the more simple hypothesis of an Y(X) chromosome located deletion of VHS resistance gene(s). Both hypotheses are compatible with our findings. In several mammalian species, a differential expression of the genome depending on the parental origin is described (Soller 1988). In several cases the observed genomic imprinting caused expression of the paternal genome only. A predominant role of the sire was found by Withler (1987) in chinook salmon (*Oncorhynchus tshawytscha*) for sur-

vival to an unknown agent. In backcrosses between splake (*Salvelinus fontinalis* × *S. namaycush* hybrid) and lake char, incidence of bluesac disease was considered to be a trait of the male splake parent (Ihssen 1978). New challenge experiments will include histological sex determination of challenged F1 offspring in order to help clarify the mechanism by which paternal resistance is mediated to the offspring.

We have found no previous evidence or reports of paternal influence on susceptibility to VHS in fish. The superior resistance to experimental VHS challenge of the triploid hybrids (rainbow trout females × coho salmon males) compared with normal, or triploid rainbow trout, described by Dorson and Chevassus (1985), is interesting. Rather than being caused by a transmission of superior genes from the resistant coho salmon sire, it might have been caused by the absence of a negative genetic influence on the offspring linked to the rainbow trout Y chromosome.

The apparently low maternal heritability of the VHS survival was not wholly unexpected. In general, traits with relation to fitness have low heritability (Falconer 1981). The possible influence of MHC polymorphism might in rainbow trout further be masked by the possibility of tetrasomic inheritance of MHC haplotypes, as suggested by Kaastrup et al. (1988), which may give rise to no less than 36 different MHC haplotype combinations (MHC genotypes) in a normal fullsib group. Since other independently segregating gene loci may also affect the VHS resistance of an individual, immunogenetically well-defined rainbow trout strains, or even clones, will be required to determine the role of MHC for VHS susceptibility. The recent report of successful production of common carp clones (Komen et al. 1990) is an encouragement for our attempts to produce clones of rainbow trout.

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Effects of Selenite and Selenate on the Growth and Motility of Seven Species of Marine Microalgae

Donald Wong and Luis Oliveira[1]

Department of Botany, University of British Columbia, Vancouver, B.C. V6T 2B1, Canada

Wong, D., and L. Oliveira. 1991. Effects of selenite and selenate on the growth and motility of seven species of marine microalgae. Can. J. Fish. Aquat. Sci. 48: 1193–1200.

Seven species of marine phytoplankters from different taxonomic divisions were tested for toxic responses to two different molecular species of selenium known to be prevalent in seawater, selenite and selenate. Selenate was slightly more toxic, although severe toxicity was only observed at high concentrations (selenate, 10^{-2} M; selenite, 10^{-3} M). At these concentrations, growth was completely or severely inhibited in most species tested. In some species that remained viable, both the percentage of motile cells and their swimming speed were drastically reduced. These cells possessed much shorter flagella compared with controls, while nonmotile cells lacked flagella altogether. Despite such striking alterations, cells of *Amphidinium carterae*, *Dunaliella tertiolecta*, and *Pavlova lutheri* showed signs of adaptation to high selenium concentrations. Lower concentrations of selenium were generally nontoxic and frequently stimulated growth. These observations suggest that both the concentration range and the length of the exposure must be considered if meaningful inferences on selenium toxicity are to be made.

Nous avons testé la réponse toxique de sept espèces de phytoplanctontes marins, appartenant à des divisions taxinomiques différentes, à deux espèces moléculaires de sélénium qui sont prévalentes dans l'eau de mer, le sélénite et le sélénate. Le sélénate était légèrement plus toxique, mais on n'a observé de toxicité grave qu'à de fortes concentrations (sélénate, 10^{-2} M; sélénite, 10^{-3} M). À de telles concentrations, la croissance était totalement ou gravement inhibée chez la plupart des espèces examinées. Chez certaines espèces qui demeuraient viables, on a observé une réduction spectaculaire du pourcentage de cellules motiles et de leur vitesse de déplacement. Ces cellules possédaient des flagelles beaucoup plus courts que ceux des cellules témoins, tandis que les cellules non motiles étaient totalement dépourvues de flagelles. Malgré des modifications aussi frappantes, les cellules d'*Amphidinium carterae*, de *Dunaliella tertiolecta* et de *Pavlova lutheri* montraient des signes d'adaptation aux fortes concentrations de sélénium. Les concentrations faibles de sélénium n'étaient généralement pas toxiques et stimulaient fréquemment la croissance. Ces observations semblent indiquer que la plage de concentration aussi bien que la durée d'exposition doivent être examinées si l'on veut tirer des conclusions fiables sur la toxicité du sélénium.

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Selenium is a minor metal or metalloid (Wilbur 1983) present in seawater in the range of 10^{-8}–10^{-10} M (Robberecht and van Grieken 1982). It is both a toxicant at high concentrations and an essential trace element at low concentrations to both animals and plants (World Health Organization 1987). Some plants are able to accumulate large amounts of selenium without showing toxic effects, while others show chlorosis and are inhibited in growth (Hurd-Karrer 1935). Many phytoplankters require selenium for growth (Harrison et al. 1988). Selenium was shown to be a cofactor for glutathione peroxidase activity in at least one diatom (Price and Harrison 1988). Selenium-deficient diatoms are elongated with altered membrane systems, with large amounts of vesicular and lipid inclusions (Doucette et al. 1987).

Recently, high concentrations of selenium found in the Kesterson Reservoir and Wild Life Refuge in California, possibly from the coastal foothills and irrigation waters, have raised concerns about toxic effects on animals in the area (Marshall 1985; Presser and Ohlendorf 1987; Ohlendorf et al. 1987). The mining, milling, smelting, and refining of metals, the manufacturing of glass, electronics components, and dyes (World Health Organization 1987), garbage incineration (Ericzon et al. 1989), coal fly ash (Brown et al. 1976; Furr et al. 1979; Lemly 1985a, 1985b), and fossil fuel combustion (Cutter and Church 1986) contribute to the selenium content of oceanic surface waters, and particularly to estuarine and coastal waters (Hamilton and Buhl 1990). Short-term studies (0–15 d) of selenium toxicity have been performed on a few species of marine microalgae (Price et al. 1987; Wheeler et al. 1982), but there are no long-term studies. Also, the possibility of adaptation to high concentrations of selenium has not been investigated. In this study, we examine the effects (up to 2 mo) of selenium exposure in seven species of marine microalgae belonging to six taxonomic divisions (classes), using the two most prevalent forms of selenium in seawater, selenite and selenate.

Materials and Methods

Stock cultures of the seven species of algae (Table 1) were maintained as previously described by Antia and Cheng (1975). Extended periods of incubation (up to 60 d) with 10^{-4} M selenite of 10^{-3} M selenate were utilized to study algal adaptation

[1]Author to whom correspondence should be addressed.

TABLE 1. Species of microalgae used in the present study.

Class and species	Strain/clone (isolator/supplier/source)
Cyanophyceae	
Agemenellum quadruplicatum	Van Baalen, ATCC No. 27264
Dinophyceae	
Amphidinium carterae	J. McLachlan, Guillard, clone "Amphi 1"
Eustigmatophyceae	
Nannochloropsis oculata	Leftley, SMBA No. 66
Pyrmnesiophyceae	
Isochrysis galbana	Leftley, SMBA No. 58
Pavlova lutheri	Leftley, SMBA No. 261
Bacillariophyceae	
Chaetoceros vixvisibilis	North East Pacific Culture Collection (Dep. of Oceanography, UBC)
Chlorophyceae	
Dunaliella tertiolecta	Woods Hole Oceanographic Institute

TABLE 2. Growth of microlagae in selenite. *a*, adaptation period (d); *b*, exponential growth rate (d^{-1}); *c*, maximum yield ($OD_{600\ nm}$); NG, no growth; NM, values too low or inadequate for measurement with confidence. Values in parentheses represent the percentages with respect to the control.

	Selenite concentration (M) added to growth medium														
	10^{-3}			10^{-4}			10^{-5}			10^{-6}			10^{-7}		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Agemenellum quadruplicatum	NG	NM	NM	9	0.029 (80)	0.49 (86)	8	0.024 (90)	0.56 (98)	7	0.030 (82)	0.54 (95)	7	0.044 (121)	0.53 (94)
Amphidinium carterae	7	0.028 (60)	0.48 (80)	7	0.034 (71)	0.53 (89)	11	0.049 (104)	0.54 (91)	11	0.048 (102)	0.57 (95)	11	0.048 (102)	0.56 (94)
Nannochloropsis oculata	18	0.045 (94)	1.30 (93)	2	0.056 (115)	1.33 (95)	2	0.50 (103)	1.47 (105)	2	0.50 (103)	1.40 (100)	2	0.051 (104)	1.22 (87)
Isochrysis galbana	4	0.026 (58)	0.50 (66)	4	0.033 (72)	0.52 (69)	4	0.055 (120)	0.86 (115)	4	0.051 (111)	0.82 (109)	4	0.047 (104)	0.79 (106)
Pavlova lutheri	25	0.023 (53)	0.58 (57)	10	0.030 (70)	0.80 (79)	9	0.049 (114)	0.83 (81)	9	0.069 (160)	1.06 (104)	9	0.074 (172)	1.23 (121)
Chaetoceros vixvisibilis	4	0.012 (76)	0.044 (42)	4	0.015 (94)	0.082 (79)	4	0.017 (109)	0.097 (94)	4	0.019 (120)	0.111 (106)	4	0.017 (110)	0.11 (104)
Dunaliella tertiolecta	2	0.047 (54)	0.33 (24)	2	0.060 (69)	0.53 (38)	2	0.078 (90)	1.30 (95)	2	0.073 (84)	1.30 (95)	2	0.078 (90)	1.33 (98)

(Stockner and Antia 1976). Growth tests were conducted in culture tubes at 18°C under continuous illumination (95–100 $\mu E \cdot m^{-2} \cdot s^{-1}$) from cool-white fluorescent lamps. Small aliquots (4 mL) of medium (Oliveira and Antia 1984), with sodium selenite (Na_2SeO_3) or sodium selenate (Na_2SeO_4), were added to 0.2-mL aliquots of a species stock culture. Selenite was used in the growth medium at concentrations ranging from 10^{-3} to 10^{-7} M, while the concentration range employed for selenate was from 10^{-2} to 10^{-5} M. These concentration ranges were chosen to avoid solubility problems at higher concentrations and lack of biological activity at lower concentrations. Selenite at 10^{-2} M produced crystals in solution, while with selenate this occurred only at 10^{-1} M. Initial tests showed that no significant effects on growth were observed at concentrations below 10^{-7} M for selenite or below 10^{-5} M for selenate. Aseptic techniques were used throughout the experiment to ensure sterility of the cultures. Growth was monitored three times each week by measuring optical density at 600 nm after brief vortex mixing. Exponential growth was calculated from the plots (region of maximum sloping) of turbidity increase with time and expressed as the percentage of corresponding growth obtained in each control without selenium. Maximum yield refers to the maximum number of cells per millilitre reached by the culture when entering stationary growth (Cheng and Antia 1970).

Culture samples were videotaped with a Zeiss photomicroscope to determine swimming speed and motility. By taking photographs of playbacks, of 1 s duration, tracks of cells were obtained which were then measured using a Kontron image analysis computer system. Distances were measured as micrometres travelled per second.

For scanning electron microscopy, cells were concentrated by gentle centrifugation and fixed, for 2 h, with 1% (v/v) OsO_4

TABLE 3. Growth of microalgae in selenate. *a*, adaptation period (d); *b*, exponential growth rate (d^{-1}); *c*, maximum yield ($OD_{600\ nm}$); NG, no growth; NM, values too low or inadequate for measurement with confidence. Values in parentheses represent the percentages with respect to the control.

	Selenate concentration (M) in growth medium											
	10^{-2}			10^{-3}			10^{-4}			10^{-5}		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Agemenellum quadruplicatum	NG	NM	NM	18	0.023 (64)	0.39 (69)	11	0.031 (85)	0.55 (96)	4	0.028 (78)	0.550 (97)
Amphidinium carterae	NG	NM	NM	4	0.014 (29)	0.30 (50)	4	0.040 (83)	0.51 (85)	4	0.044 (94)	0.580 (98)
Nannochloropsis oculata	NG	NM	NM	NG	NM	NM	2	0.045 (93)	1.50 (107)	2	0.052 (107)	1.400 (100)
Isochrysis galbana	NG	NM	NM	28	0.030 (66)	0.61 (82)	4	0.053 (117)	0.81 (108)	4	0.052 (114)	0.820 (109)
Pavlova lutheri	27	0.019 (44)	0.56 (55)	9	0.042 (99)	0.89 (87)	9	0.060 (140)	1.07 (105)	9	0.067 (157)	1.200 (118)
Chaetoceros vixvisibilis	NG	NM	NM	NG	NM	NM	4	0.012 (75)	0.079 (76)	4	0.016 (100)	0.107 (103)
Dunaliella tertiolecta	NG	NM	NM	2	0.079 (91)	1.20 (88)	2	0.072 (83)	1.37 (100)	2	0.076 (87)	1.400 (102)

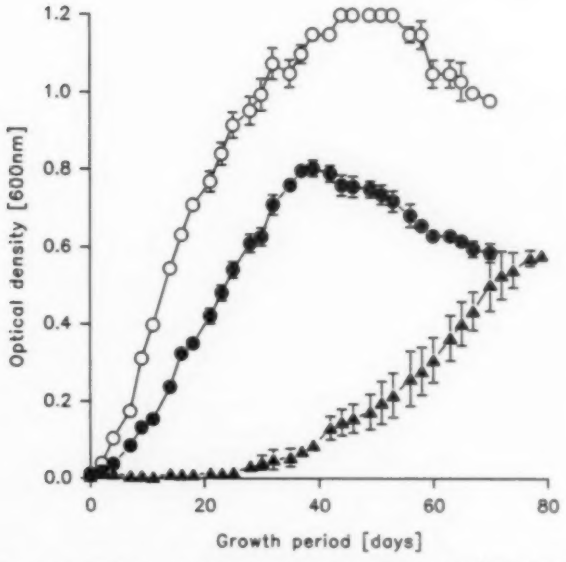

FIG. 1. Growth curves of *Pavlova lutheri* exposed to 10^{-3} M (▲) and 10^{-4} M (●) selenite and control without selenium addition (○). Vertical bars indicate the standard error of the mean; $n = 3$.

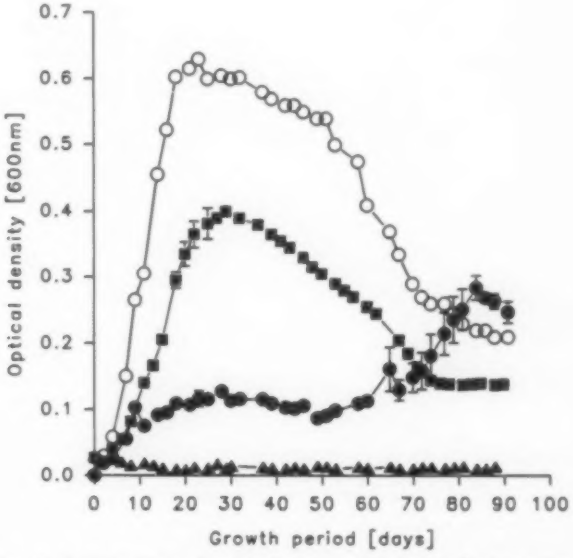

FIG. 2. Growth curve of *Amphidinium carterae* in 10^{-3} M selenate without (●) and with (■) previous adaptation, and in 10^{-2} M selenate with prior adaptation to 10^{-3} M selenate (▲). Corresponding control growth without selenium addition is also shown (○). Vertical bars indicate the standard error of the mean; $n = 3$.

in 0.1 M (w/v) sodium cacodylate, pH 7.4. Fixed cells were dehydrated in a graded ethanol series, dried to critical point, gold coated, and observed in a Cambridge Stereoscan 250T microscope.

For transmission electron microscopy, centrifuged samples were fixed, for 4 h, at 4°C with a mixture of 2% (v/v) glutaraldehyde and 1% (v/v) OsO_4 in 0.1 M (w/v) sodium cacodylate, pH 7.4. They were then concentrated by filtration for dehydration in a graded methanol series and finally embedded in Polybed 812 (Luft 1961). Ultrathin sections were cut on a Reichert OMU-3 microtome, stained with uranyl acetate and lead citrate (Reynolds 1963), and examined on a Zeiss EM10A microscope.

Results

Growth and Yield Studies

Selenite at 10^{-3} M completely inhibited the growth of *Agemenellum quadruplicatum* (Table 2). Growth inhibition was

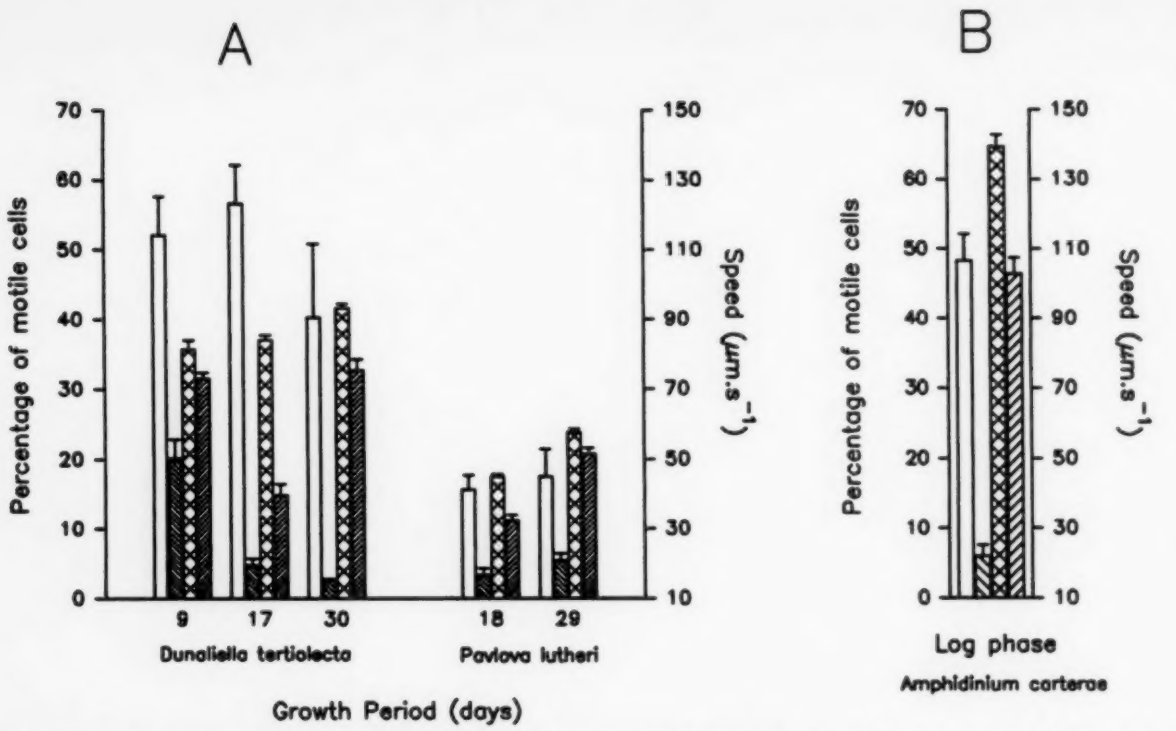

FIG. 3. Percentage and swimming speed of motile cells in cultures of (A) *Dunaliella tertiolecta* and *Pavlova lutheri* grown in 10^{-4} M selenite and (B) *Amphidinium carterae* adapted to grow in 10^{-3} M selenate. Mobility symbols: control (□), 10^{-4} M selenite (▨), 10^{-3} M selenate (◱). Swimming speed symbols: control (⊠), 10^{-4} M selenite (▩), 10^{-3} M selenate (◪). Vertical bars indicate the standard error of the mean. *Dunaliella tertiolecta*: 9 d, $n = 644$; 17 d, $n = 419$; 30 d, $n = 557$. *Pavlova lutheri*: 18 d, $n = 447$; 29 d, $n = 553$. *Amphidinium carterae*: $n = 164$.

severe in *Dunaliella tertiolecta* and major reductions in growth were also observed in *Chaetoceros vixvisibilis* and *Pavlova lutheri*. The effect upon the growth of other species (*Isochrysis galbana*, *Amphidinium carterae*, and *Nannochloropsis oculata*) was less pronounced. The reduction in maximum yield values correlated well with the reduction observed in the exponential growth rate for most of the species tested. The only noticeable exception was *Nannochloropsis oculata* with a rate of exponential growth similar to the control. The duration of the adaptation period was, in most microalgae, little affected by the concentration of selenite. The exceptions were *Nannochloropsis oculata* and *Pavlova lutheri* where increases in the adaptation period from 2 to 18 d and from 10 to 25 d respectively, were observed at 10^{-3} M selenite.

At lower concentrations of selenite, with the exception of *Dunaliella tertiolecta*, the inhibitory effects on growth were much less pronounced (Table 2). Inhibition of growth no longer occurred in *Agemenellum quadruplicatum* at 10^{-4} M selenite. The exponential growth rate of *Nannochloropsis oculata* was actually stimulated and the adaptation period dropped from 18 d at 10^{-3} M to 2 d at 10^{-4} M. Characteristic effects of selenite on phytoplanktonic organisms were exemplified by *Pavlova lutheri* (Fig. 1) where, at high concentrations (10^{-3} M), recovery from inhibition occurred after prolonged exposure. Exponential growth during the phase of recovery proceeds slower than in the control. Concentrations of 10^{-5} M or lower stimulated the growth of *Isochrysis galbana*, *Chaetoceros vixvisibilis*, and *Pavlova lutheri*. This was particularly pronounced in *Pavlova lutheri* where the exponential growth rate and maximum yield were increased to 172 and 121% of the controls at 10^{-7} M selenite, respectively (Table 2).

Selenate at 10^{-2} M completely inhibited the growth of all species tested, except *Pavlova lutheri* (Table 3), but even in this species the exponential growth rate and maximum yield were severely affected, being only 44 and 55% of the control values, respectively. Equally noticeable was the fact that growth only occurred after a rather lengthy adaptation period (27 d). *Nannochloropsis oculata* and *Chaetoceros vixvisibilis* were completely inhibited by 10^{-3} M selenate, while selenite at this concentration showed no significant impact on growth. Growth of *Agemenellum quadruplicatum* was one of the exceptions, being only partially inhibited at 10^{-3} M selenate and completely inhibited at 10^{-3} M selenite. Growth of *Isochrysis galbana* was similar in the two compounds but occurred only after a lengthy adaptation period of 28 d in selenate. Lower concentrations of selenate showed no inhibitory effects and stimulated growth in both *Isochrysis galbana* and *Pavlova lutheri* (Table 3).

The growth pattern of *Amphidinium carterae* in 10^{-3} M selenate was unusual. After an initial period of growth that lasted for 4 d and led to a maximum yield that was 20% of the control, growth stagnated (Fig. 2). This was followed, 50–60 d later, by the resumption of growth, which reached 42% of maximum exponential growth rate and 45% of maximum yield ($P < 0.05$ in both cases) of the control. This behavior did not occur in cultures previously adapted to growth in 10^{-3} M selenate. Previously adapted *Amphidinium carterae* grew

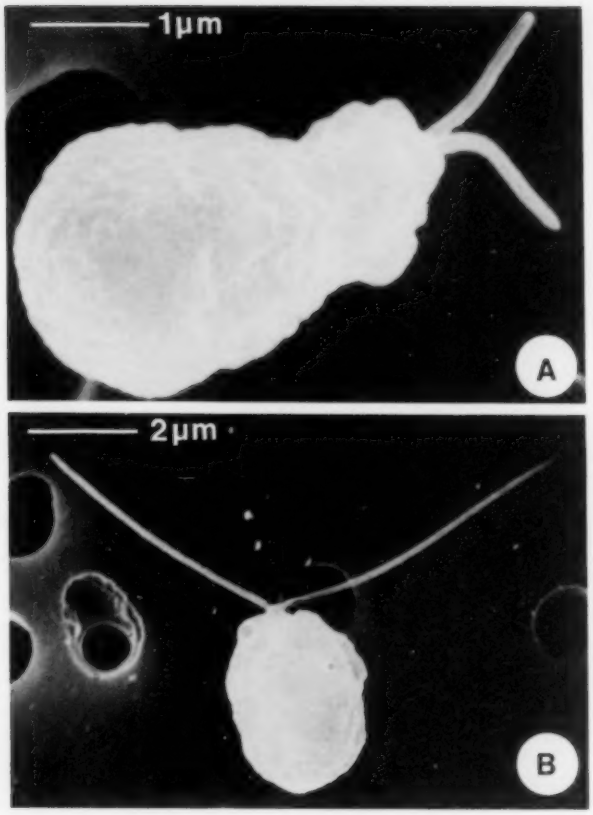

FIG. 4. Scanning electron micrograph of *Dunaliella tertiolecta* growth (A) with and (B) without selenium addition for 17 d. Note the length of the flagella in both cells.

without delay and reached 51 and 61% of maximum exponential growth rate and yield compared with the control. No growth occurred when preadapted cells were inoculated into medium with 10^{-2} M selenate (Fig. 2).

Motility and Morphological Studies

It was evident from visual inspection of the cultures that most of the microalgae grown in high concentrations of selenium settled at the bottom of the culture vessel instead of being evenly distributed or concentrated near the water/air interface as the controls were. In view of these observations, the percentage of motile cells, the swimming speed, and the flagellar structure of *Dunaliella tertiolecta* and *Pavlova lutheri* grown in 10^{-4} M selenite and *Amphidinium carterae* adapted to grow in 10^{-3} M selenate were examined. In all cases, the percentage of motile cells was significantly lower than the controls ($P < 0.05$), and so was the swimming speed of those cells that remained motile ($P < 0.05$) (Fig. 3). In *Dunaliella tertiolecta*, as the culture went from exponential (9 d) to stationary (17 d) phase, the percentage of motile cells dropped from 38.7 to 8.5% and their swimming speed from 89.8 to 47.4% of the control and then recovered to 80.8%. Recovery was also observed in *Pavlova lutheri* (Fig. 3).

Light and electron microscopy studies showed no major changes in the diameter and the internal structure of the flagella (and haptonema) in selenium-treated compared with control cells. However, 52% of the cells examined had shorter flagella (Fig. 4), while 41% lacked flagella. The differences in length were statistically significant ($P < 0.05$), with the mean flagellar length dropping to 59.4% of the control in *Dunaliella tertiolecta*. Variations in flagellar length were less pronounced in *Pavlova lutheri* and *Amphidinium carterae*. No difference was observed in the length of the haptonema of *Pavlova lutheri* in selenium-treated compared with control cultures (Table 4).

Discussion

It is evident from the present results that several marine microalgae exhibit only partial growth inhibition in selenite at almost saturation levels of concentration (10^{-3} M). One must, however, emphasize that this occurs in cells grown under nutrient-sufficient conditions. This tendency of phytoplankters to display higher resistance to selenium toxicity than other cell types, such as yeast (Brown and Smith 1979), fern (Elmore et al. 1989), several agronomic species of plants (Carlson et al. 1989), as well as a number of mammalian cultured cells (Arenholt-Bindsley et al. 1988; Morrison and Medina 1988), is supported by studies other than our own using different phytoplanktonic organisms (Sielicki and Burnham 1973; Moede et al. 1980; Wheeler et al. 1982; Gotsis 1982).

Selenate is more soluble in seawater than selenite. At its saturation level (10^{-2} M), only *Pavlova lutheri* was able to survive, although it showed signs of strong growth inhibition. All other species were inhibited at this high concentration. This is in agreement with the results obtained by other authors using different phytoplanktonic organisms (Price et al. 1987). At 10^{-4} M selenate, sharp increases in the adaptation period were required for growth resumption in *Anacystis nidulans* and *Anabaena variabilis* (Kumar and Prakash 1971), and a similar situation occurred in our studies at 10^{-3} M selenate regarding *Isochrysis galbana*. This concentration of selenate proved also to be severely inhibitory to several desmids (Sarma and Jayaraman 1984). Even lower concentrations were found to be toxic to *Chlorella pyrenoidosa* (Bennett 1988) and all the phytoplanktonic species tested by Wheeler et al. (1982). The reasons for the higher sensitivity of some phytoplankters to selenate compared with selenite at 10^{-3} M are not known but may be related to the molecular analogy of selenate to sulfate ion. Selenium transport mechanisms were shown to be differentially affected by sulfate ions (Wheeler et al. 1982; Shrift 1954; Shennan et al. 1990). Preconditioning in sulfate-containing medium protects phytoplankters against selenate toxicity (Kumar and Prakash 1971; Wheeler et al. 1982). The sulfate content of the seawater medium may play a crucial role in the cells' response to the different molecular species of selenium. One is therefore tempted to speculate that the higher tolerance of *Pavlova lutheri* to selenate may be a direct influence of the effects of sulfate upon these cells. Similarly, the uptake of phosphorus, an element not far from selenium in the periodic table, is, when in the form of phosphate, inhibited by selenate (Moede et al. 1980). The higher sensitivity to selenate may also be related to the differential uptake of these two species of selenium by cells. Indeed, selenite is absorbed in greater amounts in the duckweed *Spirodela oligorrhiza* (Butler and Peterson 1967), while the reverse is true for terrestrial plants (Ulrich and Shrift 1968). Tweedie and Segel (1970) showed that in filamentous fungi, selenate and selenite were taken up into the cells in part via the same transporter mechanism.

TABLE 4. Mean ($\bar{x}$) and standard error of the mean (SEM) of diameter and length of the flagella of *Dunaliella tertiolecta* and *Pavlova lutheri* and of the haptonema of *Pavlova lutheri* grown with and without selenite, plus diameter and length of the flagella of *Amphidinium carterae* grown with and without selenate. Observations made with a TEM, SEM, and/or light microscope.

			Diameter (μm)				Length (μm)			
			No Se added		10^{-4} M SeO_3		No Se added		10^{-4} M SeO_3	
		Days	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
Dunaliella tertiolecta	Flagella	9	22.05	0.67	21.56	0.94	15.724	0.260	16.438	0.382
		17	21.73	1.14	19.85	0.68	15.744	0.395	9.355	0.626
		30	24.35	1.77	23.59	2.79	15.417	0.230	10.625	0.429
Pavlova lutheri	Flagella	18	21.06	1.22	19.28	0.91	9.635[a]	0.462	10.286	0.608
							3.516[b]	0.651	4.323	0.268
		29	20.35	0.72	19.40	1.36	11.293	0.326	10.041	0.468
							4.358	0.584	3.795	0.641
Pavlova lutheri	Haptonema	18	12.92	2.02	12.06	1.24	1.823	0.000	2.083	0.000
		29	10.80	1.10	12.73	1.98	1.963	0.000	1.998	0.000

			Diameter (μm)				Length (μm)			
			No Se added		10^{-3} M SeO_4		No Se added		10^{-4} M SeO_4	
		Days	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
Amphidinium carterae	Flagella	Log phase	22.30	0.77	12.6	1.12	10.163	1.244	9.080	2.101

[a]Length of long anterior flagellum.
[b]Length of short posterior flagellum.

In general, the mechanism of selenium toxicity causing growth inhibition is not understood. Both selenite and selenate strongly react with sulfhydryl groups and inhibit sulfhydryl enzymes such as succinic dehydrogenase in the Krebs cycle (Ray and Ray 1975). Selenite inhibits photosynthesis and it decreases chlorophyll concentration in both phytoplankters and higher plant cells (Sielicki and Burnham 1973; Padmaja et al. 1989). Selenate also acts as an energy transfer inhibitor of photophosphorylation (Pick and Avron 1973). The inhibitory actions of selenite and selenate on the energy-producing mechanisms of plant cells certainly explain the strong growth reduction and/or total inhibition observed in microalgae at higher concentrations. Along with the reaction of selenite and selenate to sulfhydryl groups, active oxygen species (e.g. H_2O_2 and O_2^-) are also produced (Kramer and Ames 1988). These cause extensive damage to the cells, particularly to biological membranes through lipid peroxidation (Csallany and Menken 1986). Higher concentrations of selenium are also known to disturb the redox balance of the cell, affecting transport mechanisms, membrane permeability, and other membrane functions (Nebbia et al. 1990; Hewitt and Nicholas 1963). Organelle and membrane structural alterations are detectable in some of the species of microalgae used in our study, confirming that the effects of selenium toxicity are widespread (Wong and Oliveira 1991).

Selenium toxicity reduces the number of motile cells and the swimming speed in *Dunaliella tertiolecta*, *Pavlova lutheri*, and *Amphidinium carterae*. In *Dunaliella tertiolecta*, this is accompanied by a decrease in the length of the flagella without observable changes in the diameter or internal structure of these organelles. *Pavlova lutheri* showed no change in flagellar length, but in both microalgae the percentage of motile cells decreased over time. The decrease in the number of motile cells was not observed in the presence of selenate. Therefore, our results suggest that besides general effects of selenite and selenate toxicity, specialized effects associated with different forms of selenium also occur. The reasons for these selenium species dependent effects are more difficult to ascertain due to the lack of studies on the subject. However, some compounds formed by the interaction of selenite and sulfhydryl groups are known to inhibit DNA and RNA polymerase activities (Frenkel et al. 1987). A decrease in the cells' synthetic rate due to inhibition of RNA polymerase activity will adversely affect protein synthesis. This is particularly relevant for flagella and other cytoskeleton-based structures that continuously depend on a dynamic balance of protein subunit exchange to maintain their integrity and functionality (Vorobjev and Nadezhdina 1987). This problem certainly deserves further attention and it is being pursued in our laboratory.

One point that should be investigated in future studies on selenium toxicity is the ability of microalgae to adapt to higher concentrations of selenium. This is clearly seen in *Amphidinium carterae* cells treated with 10^{-3} M selenate, and it seems also to occur in cultures of *Dunaliella tertiolecta* and *Pavlova lutheri* treated with 10^{-4} M selenite. In both species, adaptation was detected by the recovery in swimming speed of cultures continuously exposed to 10^{-4} M selenite. Although these studies need to be extended to other species of microalgae, they emphasize the possibility that severe growth inhibition at high selenium concentrations may be only temporary, at least for some phytoplankters. Lower concentrations of selenium showed no effect or even proved to be stimulatory to growth.

This is in agreement with the observations of other authors that selenium is an essential trace element for growth in microalgae (Harrison et al. 1988; Price and Harrison 1988).

In conclusion, the results show that concentrations of selenium at almost saturation level are needed to cause significant growth reduction in most of the phytoplankters tested. The problem of selenite and selenate toxicity is further complicated, at least in some phytoplankters, by the possibility of adaptation to high selenium concentrations. Lower concentrations of selenium are generally nontoxic and frequently stimulate growth. Overall the observations suggest that both the concentration range and the potential for adaptation to high selenium concentrations must be considered before meaningful conclusions can be drawn from studies on selenium toxicity.

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Effects of Selenite and Selenate Toxicity on the Ultrastructure and Physiology of Three Species of Marine Microalgae

Donald Wong and Luis Oliveira[1]

Department of Botany, University of British Columbia, Vancouver, B.C. V6T 2B1, Canada

Wong, D., and L. Oliveria. 1991. Effects of selenite and selenate toxicity on the ultrastructure and physiology of three species of marine microalgae. Can. J. Fish. Aquat. Sci. 48: 1201–1211.

The main ultrastructural and physiological changes in cells of *Dunaliella tertiolecta*, *Pavlova lutheri*, and *Amphidinium carterae* treated with selenite or selenate involved the mitochondria and chloroplasts as well as the respiratory and photosynthetic rates. Other changes were observed in the nucleus, lipids, vacuoles, and nitrogen and carbon contents, but these showed greater variability among the microalgae studied. The major alterations suggested that energy-transducing systems were severely affected by selenium toxicity. These led to significant decreases or even elimination of storage products and major reductions in growth.

Les principales modifications d'ordre ultrastructurel et physiologique dans les cellules de *Dunaliella tertiolecta*, de *Pavlova lutheri* et d'*Amphidinium carterae* traitées avec du sélénite ou du sélénate touchaient les mitochondries et les chloroplastes ainsi que les taux de respiration et de photosynthèse. D'autres changements ont été observés en ce qui concerne le noyau, les lipides, les vacuoles et la teneur en azote et en carbone, mais la variabilité était grande entre les microalgues étudiées. Les principales modifications semblaient indiquer que les systèmes de transfert d'énergie étaient fortement affectés par la toxicité du sélénium. Ces modifications causaient des réductions importantes ou même l'élimination des réserves et de fortes réductions dans la croissance.

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Studies of selenium toxicity began with the discovery in the 1930's that the disorders "alkali disease, ill thrift and blind staggers" in livestock were associated with a high content of selenium in certain species of the fodder (Walker 1971). Plants containing high concentrations of selenium are known as selenium accumulators. Farmers either avoid cropping these plants or mix them with others having less selenium. The oldest case of selenium poisoning, although not identified at that time, was described by Marco Polo in one of his journeys to Asia (Giessel-Nielsen 1976). Selenium may also inhibit plant growth and cause chlorosis in leaves (Hurd-Karrer 1935).

The growth of seven species of marine microalgae was shown previously to be affected by high concentrations of selenite and selenate (Wong and Oliveira 1991). For example, at 10^{-4} M selenite, the growth of *Dunaliella tertiolecta* and *Pavlova lutheri* was severely affected, and a period of 50–60 d was required before the dinoflagellate *Amphidinium carterae* was able to sustain significant growth in 10^{-3} M selenate. Growth inhibition was accompanied in these three species by a reduction in the number of motile cells, while those that remained motile showed lower swimming speeds. This suggested that ultrastructural and physiological changes had occurred in these cells. There has been only one study on ultrastructural changes in two dinoflagellates due to selenite toxicity (Prévot and Soyer-Gobillard 1986). In this work, we report on the ultrastructure and physiology of selenium toxicity in three species of marine microalgae, *Dunaliella tertiolecta* (Chlorophyceae), *Pavlova lutheri* (Prymnesiophyceae), and *Amphidinium carterae* (Dinophyceae).

[1]Author to whom correspondence should be addressed.

Materials and Methods

Dunaliella tertiolecta, *Pavlova lutheri*, and *Amphidinium carterae* were grown in the presence of different concentrations of either selenite or selenate as described previously (Wong and Oliveira 1991). For transmission electron microscopy, samples were concentrated by gentle centrifugation and fixed, for 4 h at 4°C, in a solution of 2% (v/v) glutaraldehyde and 1% (v/v) OsO_4 in 0.1 M sodium cacodylate, pH 7.4. Dehydration was carried out in a graded methanol series, and samples were then embedded in Polybed 812 (Luft 1961). Ultrathin sections were cut on a Reichert OMU-3 microtome and examined on a Zeiss EM10A transmission electron microscope after staining with uranyl acetate and lead citrate (Reynolds 1963). Volume densities of individual organelles were calculated from electron micrographs enlarged 21 000 × for *Dunaliella tertiolecta*, 32 000 × for *Pavlova lutheri*, and 13 000 × for *Amphidinium carterae*, following the procedure described by Steer (1981). Student's *t*-test was used to compare the means of different parameters for different growth conditions.

Cell counts and cell volumes were measured on a Coulter Counter model TAII. For chlorophyll *a* and phaeophytin *a* (phaeopigment) determinations, 2 mL of cell suspensions was filtered through Whatman GF/F filters, followed immediately by immersion in 90% acetone, homogenization by grinding for 10 min, and reading of in vitro fluorescence on a Turner Designs model 10 fluorometer. A few drops of 10% HCl were added and sample readings for phaeophytin *a* concentration taken without delay.

Photosynthesis and respiration were measured at 18°C as net O_2 evolution under illumination (adjusted for respiration) or O_2

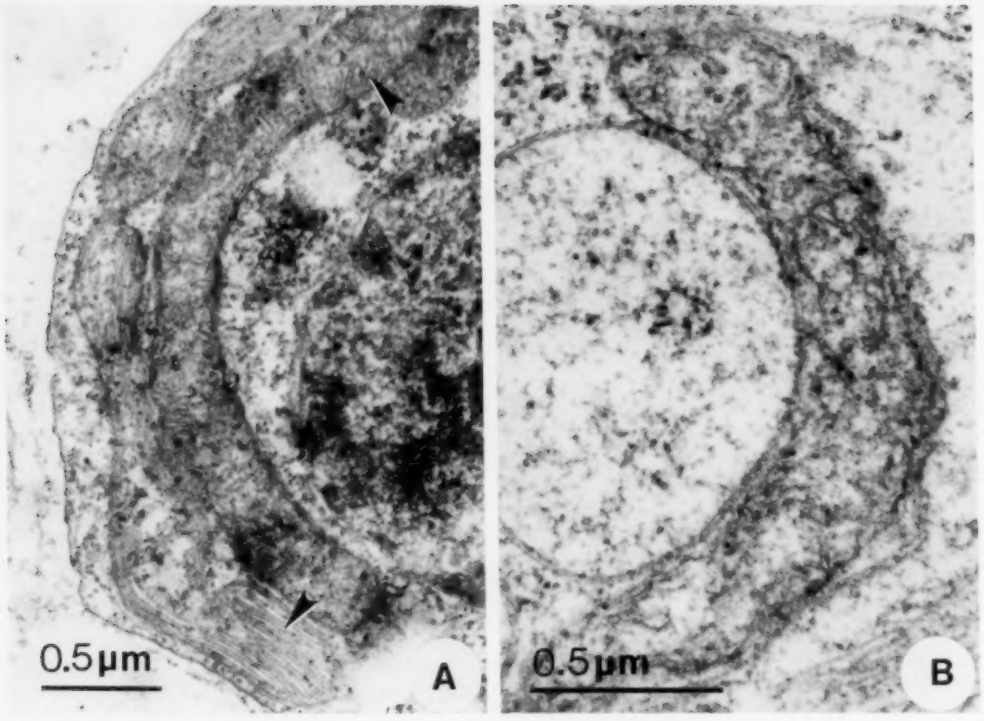

FIG. 1. Mitochondria of *Dunaliella tertiolecta* grown (A) with and (B) without 10^{-4} M selenite for 17 d. Selenium-grown cells possess long mitochondria, the cristae display unusual configurations (arrowheads), and the matrix is more dense.

consumption in darkness, respectively, using procedures described by Klut et al. (1984). Approximately 2 h of preconditioning in darkness was given to the cultures before measuring respiration, with photosynthesis measured immediately after the respiration measurements. Photosynthesis and respiration are reported as net values and expressed as micromoles O_2 evolved or consumed per minute, respectively. Particulate organic carbon and nitrogen samples were collected on 13-mm Gelman A/E filters and analyzed on a Carlo Erlba CHN analyzer. An in vivo absorption spectrum of *Dunaliella tertiolecta* was determined on a Beckman DU-62 spectrophotometer. To decrease the light scattering effects produced by the microalgal suspensions, we utilized diluted samples, a short sample path, and a detector positioned against the holding cell.

Results

The effects of 10^{-4} M selenite on *Dunaliella tertiolecta* and *Pavlova lutheri* and 10^{-3} M selenate on *Amphidinium carterae* were examined at the ultrastructural level. There was little difference in the gross ultrastructure of *Dunaliella tertiolecta* after 9 d of growth in 10^{-4} M selenite. After 17 d, selenite-grown cells had longer mitochondria with a denser matrix. The cristae also displayed unusual configurations (Fig. 1A, arrowheads). There was also a clear reduction in the size and number of cytoplasmic lipid inclusions. Chloroplasts possessed less starch ("Sth" in Fig. 2A and 2C) and the pyrenoid region tended to be smaller (compare "Pyr" in Fig. 2B and 2D). These changes were even more obvious in 30-d-old cultures when the selenite-grown cells displayed long-branched mitochondria. Chloroplasts tended to be smaller and depleted of starch deposits. Large vacuoles were present in the cytoplasm and cytoplasmic lipid inclusions were conspicuously absent (Fig. 3). After 18 d of growth in 10^{-4} M selenite, the chloroplasts of *Pavlova lutheri* were smaller and had a less-dense stroma. The mitochondria were also smaller and more numerous when compared with the control (Fig. 4). No other major ultrastructural changes were observed.

This is in contrast with the changes observed in the ultrastructure in *Dunaliella tertiolecta* cells grown in 10^{-3} M selenate. In the nucleus, the chromosomal material was less osmiophilic, and the perichromatic granules were more evident in selenium-treated than in control cells. The vacuoles were large and contained large amounts of inclusions (Fig. 5A and 5B). The thylakoids became grossly dilated producing large areas with a vesiculated appearance within chloroplasts (Fig. 5C and 5D).

Cells of *Dunaliella tertiolecta* grown in 10^{-4} M selenite for 9 or 17 d were larger than the controls ($P < 0.15$ and $P < 0.30$, respectively; Fig. 6). The process was reversed after 30 d when the control cells tended to increase in size, while those grown in selenite showed a significant reduction ($P < 0.05$). Cells of *Pavlova lutheri* grown in 10^{-4} M selenite for 18 and 29 d were also larger than the control ($P < 0.10$ and $P < 0.30$, respectively). In this instance, however, no decrease in cell size was observed with aging in selenium-treated cells. *Amphidinium carterae* grown in 10^{-3} M selenate

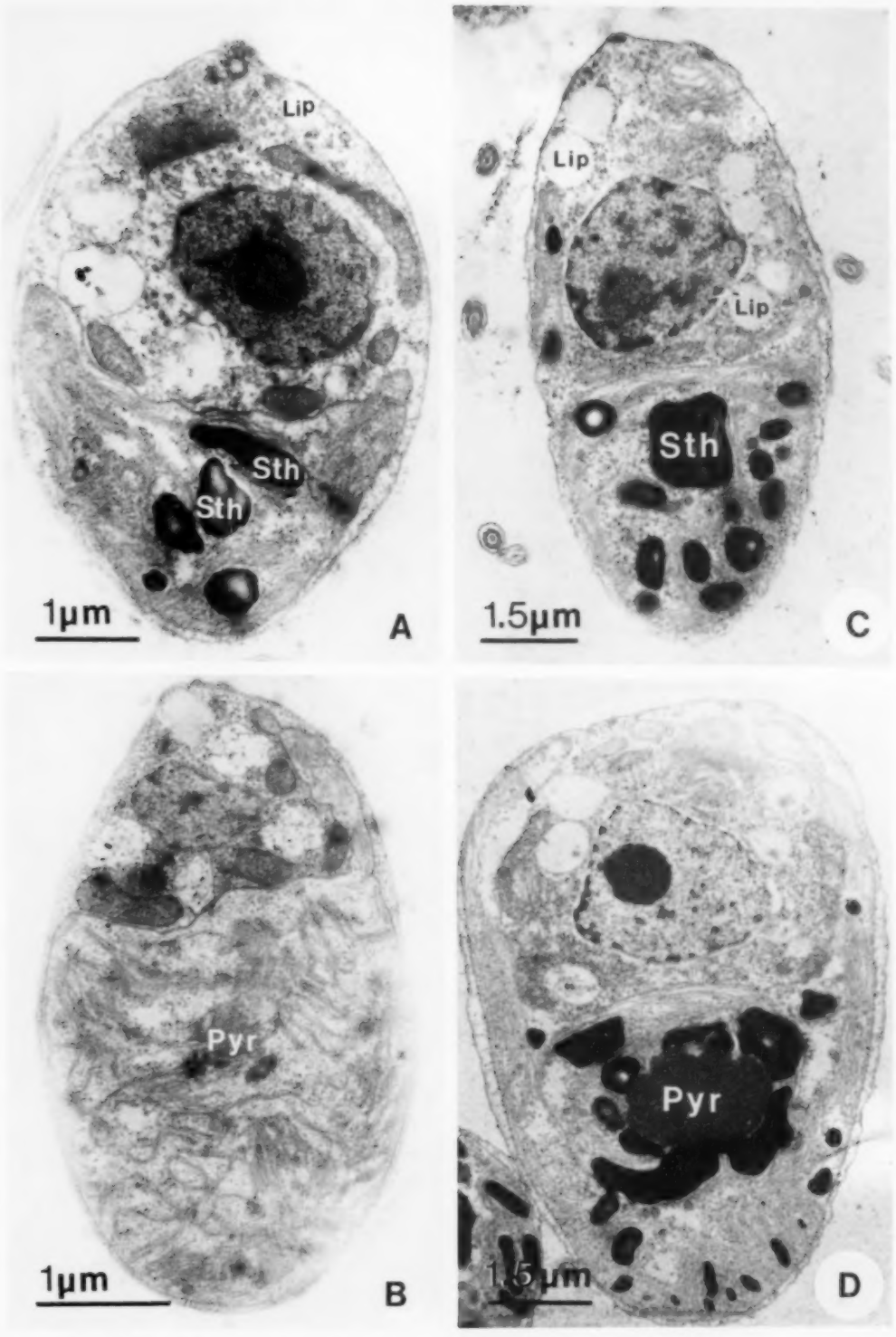

FIG. 2. *Dunaliella tertiolecta* grown (A and B) with (C and D) without 10^{-4} M selenite for 17 d. Note the smaller amount of starch (Sth) (compare Fig. 2A and 2C) and smaller pyrenoid (Pyr) (compare Fig. 2B and 2D) in selenite-grown cells. These cells are also largely devoid of lipid inclusions (Lip) (compare Fig. 2A and 2C).

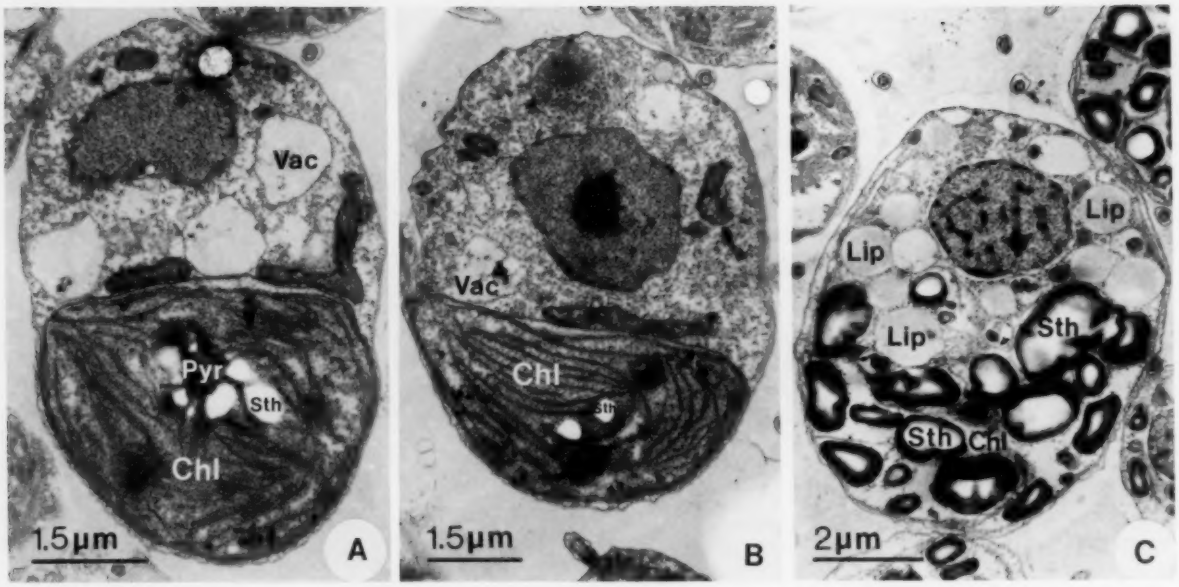

FIG. 3. *Dunaliella tertiolecta* grown (A and B) and (C) without 10^{-4} M selenite for 30 d. Large vacuoles (Vac), lack of lipid inclusions (Lip), smaller chloroplasts (Chl), pyrenoid (Pyr), and less starch (Sth) characterize the selenium-grown cells.

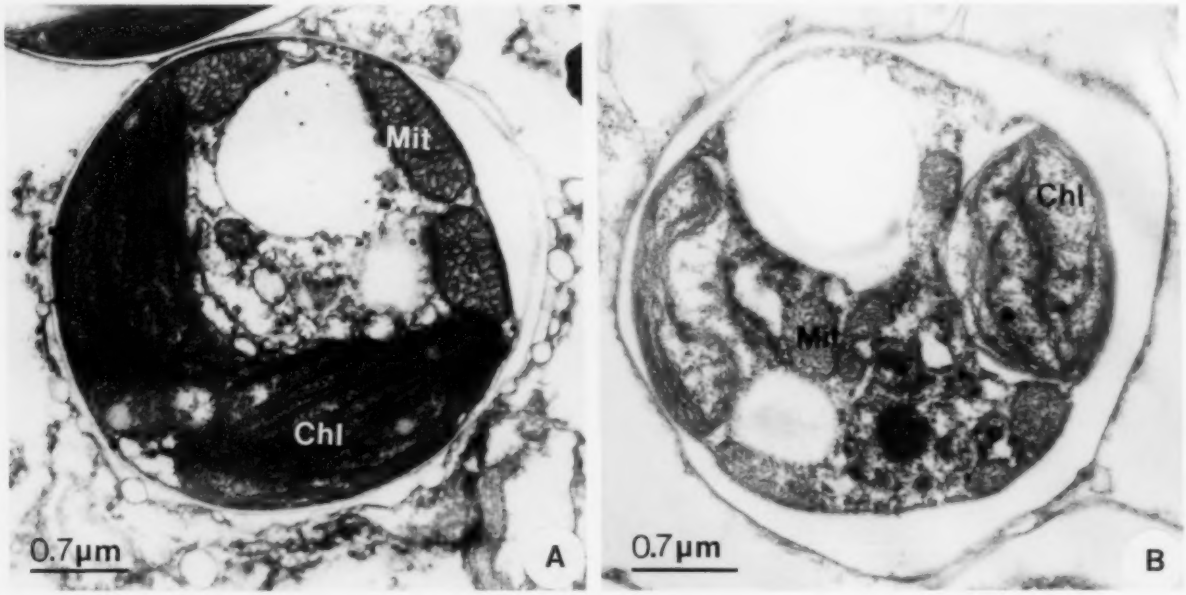

FIG. 4. *Pavlova lutheri* grown (A) without and (B) with 10^{-4} M selenite for 18 d. Note the small size of both chloroplasts (Chl) and mitochondria (Mit) in selenium-grown compared with control cells.

showed no significant changes in cell size compared with the control (Fig. 6).

Morphometric analysis, expressed as volume density, of the major organelles of *Dunaliella tertiolecta* was conducted at 9, 17, and 30 d of exposure to 10^{-4} M selenite (Fig. 7). Major differences were only observed after 17 d when the mitochondria (Mit) increased 73% in volume ($P < 0.005$). The volume of lipid inclusions (Lip) decreased 76% ($P < 0.001$). Pyrenoids (Pyr) were 90% smaller ($P < 0.005$), and there was 73% less starch (Sth; ($P < 0.001$). Overall, chloroplasts (Chl) became 13% smaller ($P < 0.001$) also compared with the control. After 30 d the mitochondrial volume was still 72% larger than the control ($P < 0.005$). The vacuolar (Vac) compartment decreased 43% ($P < 0.001$) and the volume of lipid inclusions decreased 99% ($P < 0.001$). There was no statistically significant difference in the volume of pyrenoids between

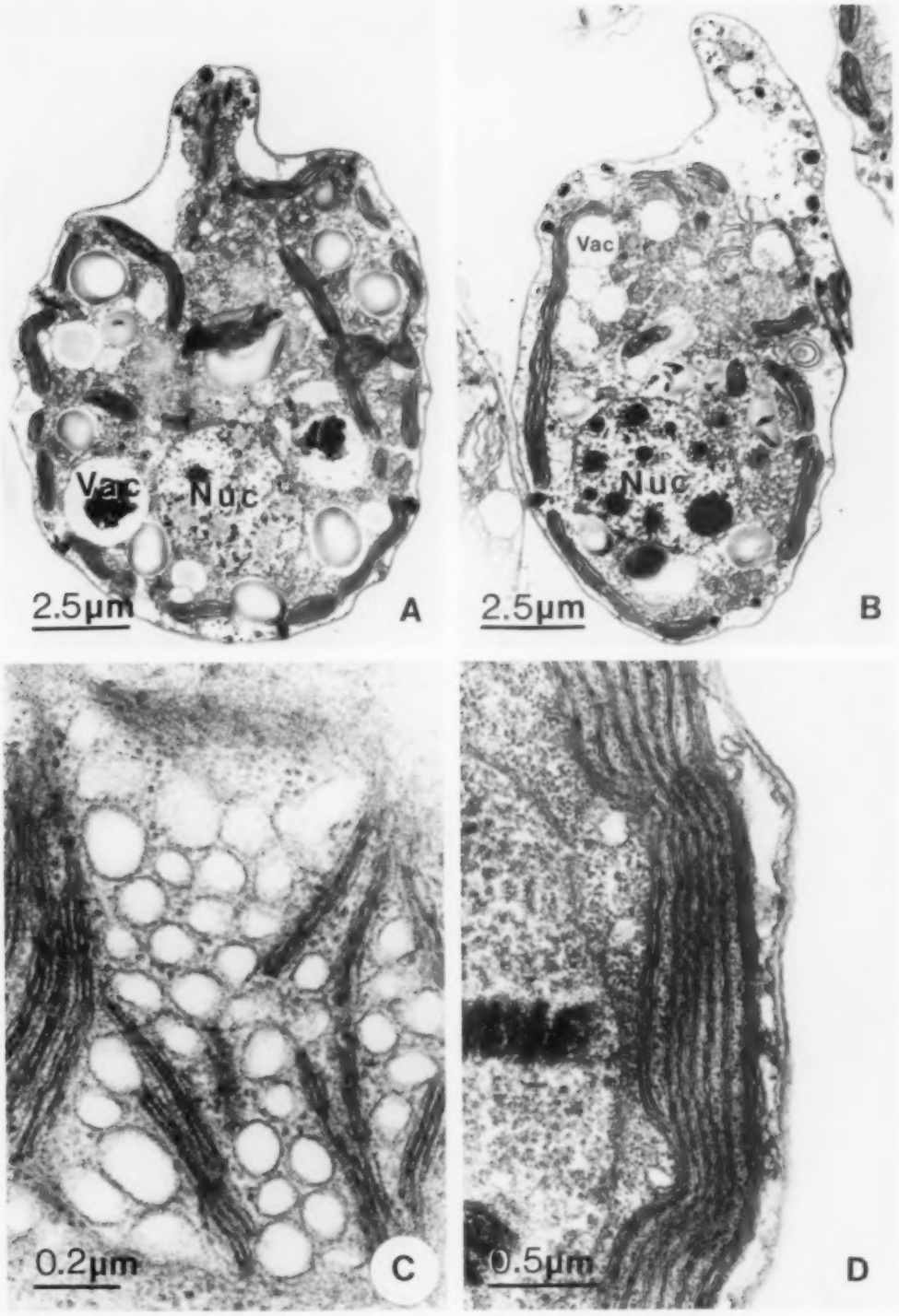

FIG. 5. *Amphidinium carterae* at log phase grown (A and C) and with (B and D) without 10^{-3} M selenate. Note the differences in the nucleus (Nuc) and vacuoles (Vac) of selenium-grown (Fig. 5A) and control cells (Fig. 5B). Note also the vesiculation of the thylakoids in selenium-grown (Fig. 5C) and control cells (Fig. 5D).

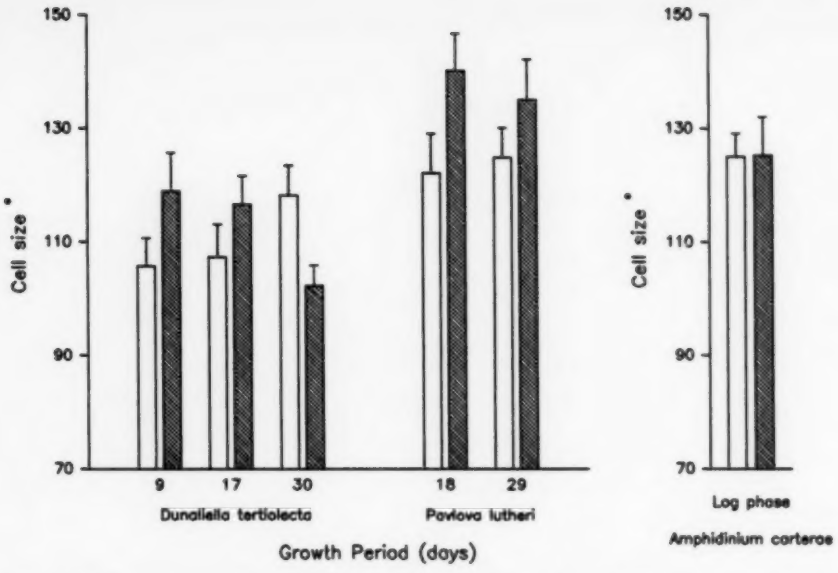

FIG. 6. Cell size, expressed as number of points over cross section, of *Dunaliella tertiolecta* and *Pavlova lutheri* after growth for various periods of time in 10^{-4} M selenite, *Amphidinium carterae* at log phase grown in 10^{-3} M selenate (▨), and growth without selenium addition (control, □). Vertical bars indicate the standard error of the mean. *Dunaliella tertiolecta*: 9 d, $n = 50$; 17 d, $n = 72$; 30 d, $n = 49$. *Pavlova lutheri*: 18 d, $n = 51$; 29 d, $n = 52$. *Amphidinium carterae*: $n = 49$.

the control and the cells grown in 10^{-4} M selenite ($P < 0.10$), although there was an 86% decrease in the volume density of starch granules ($P < 0.001$). Overall, the chloroplast volume density decreased by 19% ($P < 0.001$).

There were not nearly as many changes in *Pavlova lutheri* grown in 10^{-4} M selenite (Fig. 8). The only change observed after 18 d was in the volume density of chloroplasts that were 27% smaller ($P < 0.005$). Differences observed in other organelles proved to be statistically not significant. No further changes in volume density of most organelles were detected after 29 d of growth in selenite, although the chloroplasts remained 16% smaller ($P < 0.20$) and the mitochondria 21% larger ($P < 0.25$) than the control. With *Amphidinium carterae* grown in 10^{-3} M selenate, the sole difference found by morphometric analysis was that the volume of vacuoles with inclusions (VIn) was 140% greater than the control ($P < 0.050$) (Fig. 8). Other changes proved to be statistically not significant.

Dunaliella tertiolecta, grown in 10^{-4} M selenite for 17 d, had 20–30% less chlorophyll *a* and phaeophytin *a* than the control (Table 1). The decrease in pigmentation was observed when the pigment content was expressed per cell, per cubic micrometre of protoplast, or per cubic micrometre of chloroplast. Photosynthesis (expressed per cell or per cubic micrometre of chloroplast) was also considerably reduced under these circumstances and accounted for 70–75% of the control. Respiration was 43% less when expressed per cubic micrometre of mitochondria. However, no significant difference in respiration was observed when the values were expressed per cubic micrometre of protoplast or per cell (Table 1). The color of the *Dunaliella tertiolecta* cultures grown with 10^{-4} M selenite changed from green to a reddish brown at the end of the exponential phase. The in vivo absorption spectrum of *Dunaliella tertiolecta* at this time showed that there was a change in the shape of the spectrum between 500 and 600 nm (Fig. 9). The ratio between some of the peaks, corresponding to different pigments, also changed (Table 2). There was a 32% increase in the ratio of carotene to chlorophyll *a* at 620–630 nm ($P < 0.010$) and a 34% increase at 680 nm ($P < 0.050$). There was also a small increase in the ratio of chlorophyll *b* to chlorophyll *a* (430–440 nm) that coincided with the shift in the absorption of chlorophyll *a*. The ratio of chlorophyll *a* absorption at 430–440 and 680 nm increased 39% ($P < 0.001$). Changes in the cell size measured by Coulter Counter and in carbon and nitrogen contents were not statistically significant.

Similar physiological studies were conducted with *Pavlova lutheri* and *Amphidinium carterae*. However, in vivo absorption spectra were not taken because no changes in pigmentation were detected with selenium treatments. Chlorophyll *a* and phaeophytin *a* decreased by 40–50% in *Pavlova lutheri*, while photosynthesis decreased by 30–50% when expressed per cell, per cubic micrometre of protoplast, or per cubic micrometre of chloroplast. There was a 27% reduction in the amount of respiration expressed per cubic micrometre of mitochondria. However, the decrease was smaller and in the order of 15–17% when expressed per cell or per cubic micrometre of protoplast. The carbon and nitrogen contents of the cells were also decreased approximately 50 and 40%, respectively, when expressed per cell or per cubic micrometre of protoplast (Table 3).

Amphidinium carterae grown in 10^{-3} M selenate showed a decrease of approximately 50 and 40% in the chlorophyll *a* and phaeophytin *a* concentrations, respectively, when expressed on a cell, cubic micrometre of protoplast, or cubic micrometre of chloroplast basis. The photosynthetic rate decreased by 35%·$(\mu m^3$ chloroplast$)^{-1}$, but this value was conspicuously smaller (5–11%) when expressed per cell or per cubic micrometre of protoplast. Respiration increased by 30–40% when expressed per cell, per cubic micrometre of protoplast, or per

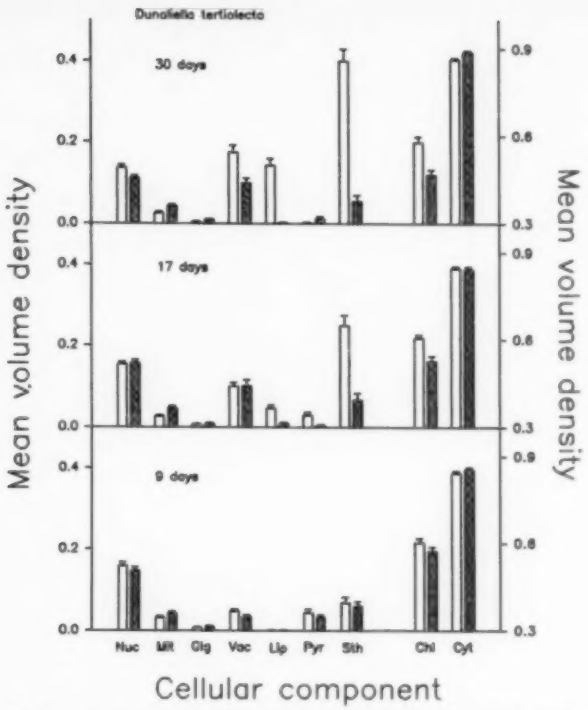

FIG. 7. Mean volume density of cell components of *Dunaliella tertiolecta* after growth for various periods of time in 10^{-4} M selenite (▨) and control growth without selenium (□). Vertical bars indicate the standard error of the mean. Nucleus (Nuc) and cytoplasm (Cyt) volume densities are expressed per cell volume. Chloroplast (Chl), mitochondria (Mit), golgi (Glg), vacuoles (Vac), and lipid inclusion (Lip) volume densities are expressed per cytoplasmic volume. Pyrenoid (Pyr) and starch (Sth) volume densities are expressed per chloroplast volume. 9 d, $n = 50$; 17 d, $n = 72$; 30 d, $n = 49$.

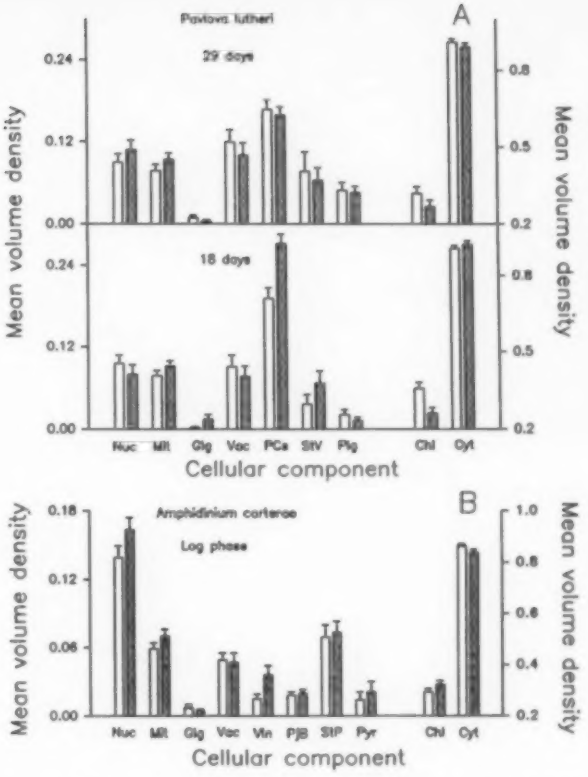

FIG. 8. Mean volume density of cell components of *Pavlova lutheri* after growth for various periods of time in 10^{-4} M selenite and *Amphidinium carterae* at log phase, adapted to grow in 10^{-3} M selenate (▨). Corresponding control growth without selenium addition is also shown (□). Vertical bars indicate the standard error of the mean. Nucleus (Nuc), cytoplasm (Cyt), and peripheral cisternae (PCs) are expressed per cell volume. Chloroplast, (Chl), mitochondria (Mit), golgi (Glg), vacuoles (Vac), storage vesicles (StV), projectile bodies (PjB), and storage products (starch and lipids (StP) are expressed per cytoplasmic volume. Plastoglobuli (Plg) and pyrenoid (Pyr) are expressed per chloroplast volume. *Pavlova lutheri*: 18 d, $n = 51$; 29 d, $n = 52$. *Amphidinium carterae*: $n = 49$.

cubic micrometre of mitochondria. The amount of carbon per cell decreased 6% ($P < 0.001$), although there was no statistically significant decrease in the amount of carbon per cubic micrometre of protoplast due to little variation in cell size measured by the Coulter Counter. The amount of nitrogen per cell and per cubic micrometre of protoplast decreased 18% ($P < 0.050$) and 13% ($P < 0.050$), respectively (Table 4).

Discussion

The main ultrastructural and physiological changes observed in cells treated with high selenite and selenate concentrations involved the mitochondria and chloroplasts as well as the respiratory and photosynthetic rates. Other changes were observed in the nucleus, lipids, vacuoles, cell size, and nitrogen and carbon contents, but these showed greater variability among the organisms tested.

Mitochondria were most affected in *Dunaliella tertiolecta* after 17 and 30 d of growth in 10^{-4} M selenite. An increase in length was accompanied by an increase in the density of the matrix and unusual cristae arrangement. This coincided in 17-d-old cultures with a lower respiratory rate. However, since the overall volume of the mitochondrial compartment increased by 73%, the total amount of respiration expressed per volume protoplast or per cell was no different from the cells grown without selenium addition. This compensation strategy for the decrease in respiration per volume of mitochondria due to high selenium concentration was also observed in *Pavlova lutheri*. The 18% increase observed in mitochondrial volume lessened the drop in the respiratory rate from 27% to as low as 15% when expressed per cell or per volume protoplast.

Giant mitochondria with dilated cristae were observed in *Prorocentrum micans* grown in selenite at the 50–100 ppm (10^{-3}–10^{-4} M) range, but not in *Crypthecodinium cohnii* (Prévot and Soyer-Gobillard 1986). The increase in the density of the mitochondrial matrix has not been previously observed with growth in selenium, but it occurs with chloramphenicol inhibition of organelle protein synthesis (Smith et al. 1970; Smith-Johannsen and Gibbs 1972). It is well documented that selenium inhibits cytoplasmic protein synthesis (Gruenwedel and Cruickshank 1979; Lewko et al. 1985). Nonetheless, it is unlikely that protein synthesis inhibition in the species tested here was the major cause of mitochondrial alterations. Indeed, the increases observed both in the size and volume of the mito-

TABLE 1. Mean ($\bar{x}$) and standard error of the mean (SEM) of physiological activities of *Dunaliella tertiolecta* grown with and without selenite.

Parameter	No Se added		10^{-4} M SeO_3	
	$\bar{x}$	SEM	$\bar{x}$	SEM
Cell size (μm^3)	83.239	1.497	85.776	1.119
Chlorophyll *a*				
$\mu g\ cell^{-1}$	8.913×10^{-7}	0.786×10^{-7}	6.140×10^{-7}	0.735×10^{-7}
$\mu g\ (\mu m^3\ protoplast)^{-1}$	1.075×10^{-8}	0.127×10^{-8}	7.181×10^{-9}	1.016×10^{-9}
$\mu g\ (\mu m^3\ chloroplast)^{-1}$	2.097×10^{-8}	0.249×10^{-8}	1.621×10^{-8}	0.229×10^{-8}
Phaeophytin a				
$\mu g\ cell^{-1}$	4.657×10^{-3}	0.438×10^{-3}	3.175×10^{-3}	0.331×10^{-3}
$\mu g\ (\mu m^3\ protoplast)^{-1}$	5.617×10^{-5}	0.698×10^{-5}	3.712×10^{-5}	0.468×10^{-5}
$\mu g\ (\mu m^3\ chloroplast)^{-1}$	1.096×10^{-4}	0.136×10^{-4}	8.378×10^{-5}	1.057×10^{-5}
Photosynthesis (O_2 evolved)				
$\mu mol\ cell^{-1} \cdot min^{-1}$	3.026×10^{-11}	0.626×10^{-11}	8.308×10^{-12}	0.040×10^{-12}
$\mu mol\ (m^3\ protoplast)^{-1} \cdot min^{-1}$	3.663×10^{-13}	0.866×10^{-13}	9.689×10^{-14}	0.168×10^{-14}
$\mu mol\ (\mu m^3\ chloroplast^{-1} \cdot min^{-1}$	7.147×10^{-13}	1.689×10^{-13}	2.187×10^{-13}	0.038×10^{-13}
Respiration (O_2 consumed)				
$\mu mol\ cell^{-1} \cdot min^{-1}$	6.725×10^{-12}	0.342×10^{-12}	6.813×10^{-12}	0.342×10^{-12}
$\mu mol\ (\mu m^3\ protoplast)^{-1} \cdot min^{-1}$	8.074×10^{-14}	0.163×10^{-14}	7.938×10^{-14}	0.223×10^{-14}
$\mu mol\ (\mu m^3\ mitochondria)^{-1} \cdot min^{-1}$	3.666×10^{-12}	0.074×10^{-12}	2.090×10^{-12}	0.059×10^{-12}
Carbon				
$mg\ cell^{-1}$	2.323×10^{-8}	0.106×10^{-8}	2.213×10^{-8}	0.193×10^{-8}
$mg\ (\mu m^3\ protoplast)^{-1}$	2.789×10^{-10}	0.041×10^{-10}	2.586×10^{-10}	0.282×10^{-10}
Nitrogen				
$mg\ cell^{-1}$	3.885×10^{-9}	0.704×10^{-9}	4.407×10^{-9}	0.616×10^{-9}
$mg\ (\mu m^3\ protoplast)^{-1}$	4.697×10^{-11}	0.990×10^{-11}	5.156×10^{-11}	0.832×10^{-11}

chondria without signs of swelling suggest that these alterations are more likely to require synthesis of new material rather than inhibition. Hackenbrock (1966, 1968) considered this type of ultrastructural change a manifestation of transitions occurring between respiratory states, paralleled by the inhibition of oxidative phosphorylation. It had also been suggested that selenium effects on sulfhydryl groups affect oxidative phosphorylation. In fact, selenium exerts an inhibitory effect on many dehydrogenating enzymes and urease. This was proposed to be due to the blockage of sulfhydryl groups essential to oxidative processes (Underwood 1977). This could explain the lower respiratory rates exhibited by the microalgae in the presence of selenium. These findings seem to be in conflict with the increase in respiration observed in *Amphidinium carterae* exposed to selenate. However, *Amphidinium carterae* cells also displayed an increase in respiration when exposed to higher concentrations of fluoride (Klut et al. 1984).

The chloroplast was the other organelle severely affected by selenium. Both *Dunaliella tertiolecta* and *Pavlova lutheri* showed a reduction in chloroplast volume in association with decreases in the amounts of chlorophyll *a*, phaeophytin *a*, and photosynthesis when grown in 10^{-4} M selenite. *Dunaliella tertiolecta* also exhibited a smaller pyrenoid and less starch. The decrease in both the chloroplast volume and rate of photosynthesis will certainly reduce the amount of energy available to the cells. This is consistent with the observations of other authors showing that photophosphorylation is inhibited by selenium (Pick and Avron 1973) and with our own observations that selenium treatments lead to the depletion of storage products, such as starch and cytoplasmic lipid inclusions. This is further corroborated by the analysis of the nitrogen and carbon contents of the cells.

When the blue-green alga *Phormidinium luridum* was grown in 10^{-2}–10^{-5} M selenite, there was a decrease in the amount of chlorophyll *a* and photosynthesis (Sielicki and Burnham 1973). The seedlings of mung bean (*Phaseolus vulgaris*) were affected by 10^{-5} M selenium. The reduction in chlorophyll was found to be paralleled by the inhibition of an enzyme proposed to be involved in chlorophyll biosynthesis (Padmaja et al. 1989). In *Dunaliella tertiolecta* and *Pavlova lutheri*, both chlorophyll *a* and phaeophytin *a* decreased approximately by the same amount. This suggests that a massive breakdown of chlorophyll *a* to phaeophytin *a* was not occurring, but the inhibition of chlorophyll synthesis was likely the cause of the reduction in chlorophyll *a* concentration. However, with *Amphidinium carterae* grown in 10^{-3} M selenate, the decrease in chlorophyll *a* was 10% greater than that of phaeophytin *a*. This is indicative either of an increase in chlorophyll *a* breakdown or a reduction in phaeophytin *a* breakdown. These observations further reinforce those of a previous work showing that cells exhibit specific toxicity responses to different forms of selenium (Wong and Oliveira 1991).

In vivo absorption spectra of *Dunaliella tertiolecta* show a decrease in the amount of chlorophyll *a* absorption relative to carotenoid and a decrease in the absorbance of chlorophyll *a* at 680 nm that is further accentuated at 430–440 nm. Overall, there seemed to be a shift from absorption at longer wavelengths to shorter ones. The reason for this is unclear. However, given that in *Amphidinium carterae* grown in 10^{-3} M selenate, many thylakoids were dilated, and given that selenium is known to react with sulfhydryl groups to form active oxygen species which can cause lipid peroxidation (Kramer and Ames 1988), it is conceivable that thylakoid damage could lead to shifts in the in vivo absorption spectra.

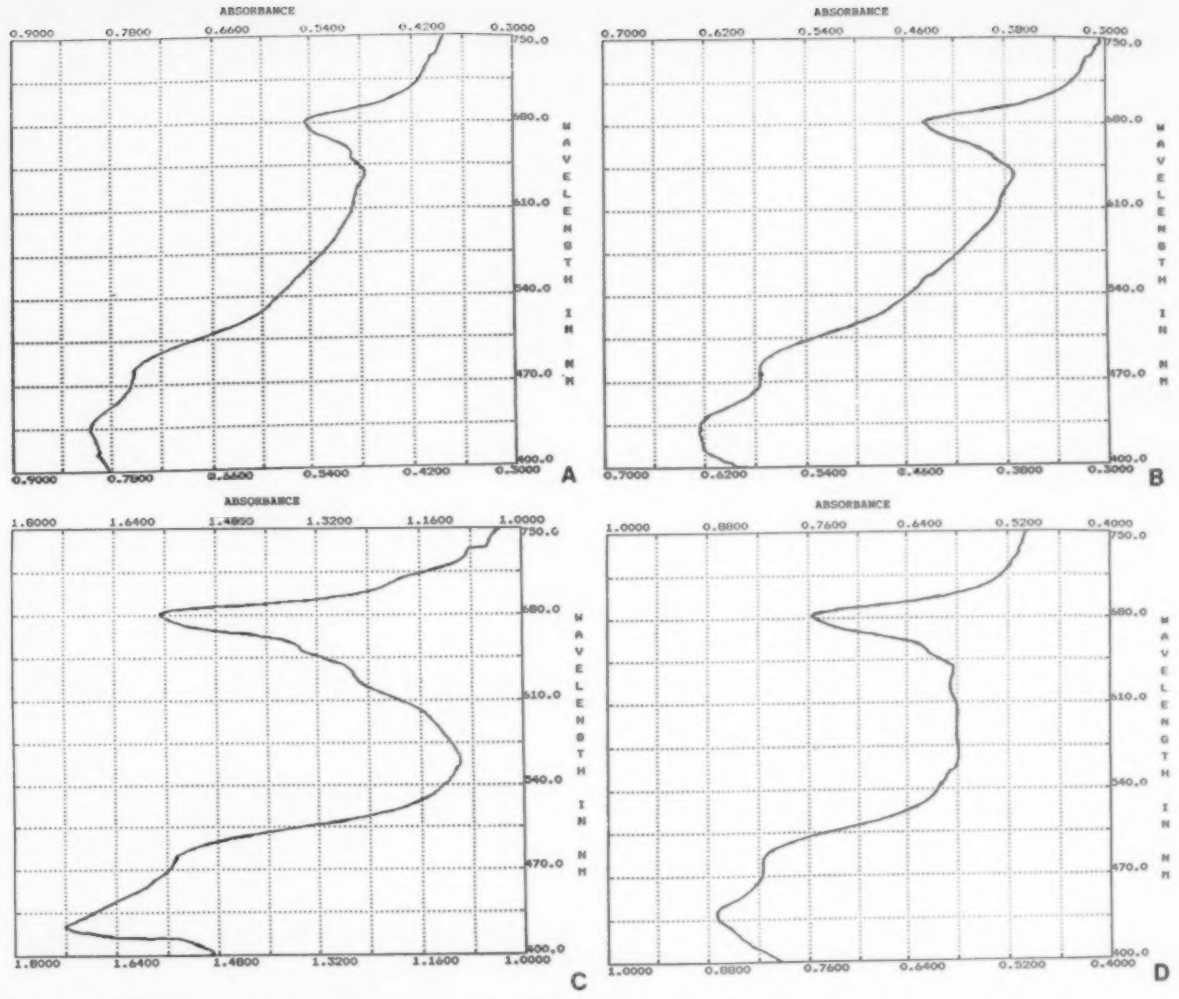

FIG. 9. In vivo absorption spectra of *Dunaliella tertiolecta* grown in (A and B) 10^{-4} M selenite or (C and D) without selenium addition for 17 d. Note the difference in the shape of the absorption curves between 500 and 600 nm. $n = 4$.

TABLE 2. Mean ($\bar{x}$) and standard error mean (SEM) of the absorption by different pigments from two in vivo absorption spectra replicate measurements of *Dunaliella tertiolecta* grown with and without selenite.

	17-d-old cells			
	No Se added		10^{-4} M SeO_3	
Ratio	$\bar{x}$	SEM	$\bar{x}$	SEM
Chl *a* (430–440 nm) to carotene (480 nm)	1.065	0.052	1.083	0.083
Chl *a* (430–440 nm) to Chl *a* (620–630 nm)	1.277	0.088	1.715	0.151
Chl *a* (430–440 nm) to Chl *b* (650 nm)	1.304	0.040	1.474	0.051
Chl *a* (430–440 nm) to Chl *a* (680 nm)	1.094	0.001	1.526	0.001
Carotene (480 nm) to Chl *a* (620–630 nm)	1.198	0.024	1.582	0.018
Carotene (480 nm) to Chl *b* (650 nm)	1.230	0.098	1.373	0.152
Carotene (480 nm) to Chl *a* (680 nm)	1.030	0.050	1.381	0.072
Chl *a* (620–630 nm) to Chl *b* (650 nm)	1.029	0.102	0.869	0.106
Chl *a* (620–630 nm) to Chl *a* (680 nm)	0.861	0.059	0.873	0.055
Chl *b* (630 nm) to Chl *a* (680 nm)	0.840	0.026	1.013	0.060

TABLE 3. Mean ($\bar{x}$) and standard error of the mean (SEM) of physiological activities of *Pavlova lutheri* grown with and without selenite.

Parameter	No Se added		10^{-4} M SeO_3	
	$\bar{x}$	SEM	$\bar{x}$	SEM
Cell size (μm^3)	38.423	0.495	39.374	0.492
Chlorophyll a				
$\mu g\ cell^{-1}$	1.001×10^{-6}	0.222×10^{-6}	5.015×10^{-7}	0.225×10^{-7}
$\mu g\ (\mu m^3\ protoplast)^{-1}$	2.601×10^{-8}	0.555×10^{-8}	1.273×10^{-8}	0.032×10^{-8}
$\mu g\ (\mu m^3\ chloroplast)^{-1}$	5.075×10^{-8}	1.084×10^{-8}	2.873×10^{-8}	0.072×10^{-8}
Phaeophytin *a*				
$\mu g\ cell^{-1}$	4.437×10^{-3}	0.968×10^{-3}	2.268×10^{-3}	0.105×10^{-3}
$\mu g\ (\mu m^3\ protoplast)^{-1}$	1.153×10^{-4}	0.243×10^{-4}	5.757×10^{-5}	0.152×10^{-5}
$\mu g\ (\mu m^3\ chloroplast)^{-1}$	2.250×10^{-4}	0.473×10^{-4}	1.299×10^{-4}	0.034×10^{-4}
Photosynthesis (O_2 evolved)				
$\mu mol\ cell^{-1} \cdot min^{-1}$	4.068×10^{-11}	1.846×10^{-11}	2.195×10^{-11}	0.837×10^{-11}
$\mu mol\ (\mu m^3\ protoplast)^{-1} \cdot min^{-1}$	1.055×10^{-14}	0.472×10^{-14}	5.535×10^{-13}	2.016×10^{-13}
$\mu mol\ (\mu m^3\ chloroplast)^{-1} \cdot min^{-1}$	3.275×10^{-12}	1.465×10^{-12}	2.302×10^{-12}	0.839×10^{-12}
Respiration (O_2 consumed)				
$\mu mol\ cell^{-1} \cdot min^{-1}$	2.135×10^{-11}	1.212×10^{-11}	1.816×10^{-11}	0.458×10^{-11}
$\mu mol\ (\mu m^3\ protoplast)^{-1} \cdot min^{-1}$	5.531×10^{-13}	3.108×10^{-13}	4.591×10^{-13}	1.072×10^{-13}
$\mu mol\ (\mu m^3\ mitochondria)^{-1} \cdot min^{-1}$	7.938×10^{-12}	4.461×10^{-12}	5.786×10^{-12}	1.588×10^{-12}
Carbon				
$mg\ cell^{-1}$	2.307×10^{-8}	0.397×10^{-8}	3.476×10^{-8}	0.061×10^{-8}
$mg\ (\mu m^3\ protoplast)^{-1}$	5.997×10^{-10}	0.985×10^{-10}	8.829×10^{-10}	0.022×10^{-10}
Nitrogen				
$mg \cdot cell^{-1}$	4.306×10^{-9}	1.039×10^{-9}	5.969×10^{-9}	0.194×10^{-9}
$mg\ (\mu m^3\ protoplast)^{-1}$	1.119×10^{-10}	0.261×10^{-10}	1.517×10^{-10}	0.080×10^{-10}

TABLE 4. Mean ($\bar{x}$) and standard error of the mean (SEM) of physiological activities of log-phase *Amphidinium carterae* grown with and without selenate.

Parameter	No Se added		10^{-4} M SeO_4	
	$\bar{x}$	SEM	$\bar{x}$	SEM
Cell size (μm^3)	643.263	5.247	606.501	1.982
Chlorophyll *a*				
$\mu g\ cell^{-1}$	1.283×10^{-7}	0.309×10^{-7}	5.999×10^{-6}	2.265×10^{-6}
$\mu g\ (\mu m^3\ protoplast)^{-1}$	1.993×10^{-8}	0.474×10^{-8}	9.991×10^{-9}	3.788×10^{-9}
$\mu g\ (\mu m^3\ chloroplast)^{-1}$	7.872×10^{-8}	1.873×10^{-8}	3.677×10^{-8}	1.405×10^{-8}
Phaeophytin *a*				
$\mu g\ cell^{-1}$	2.872×10^{-2}	0.498×10^{-2}	1.787×10^{-2}	0.061×10^{-2}
$\mu g\ (\mu m^3\ protoplast)^{-1}$	4.462×10^{-5}	0.760×10^{-5}	2.947×10^{-5}	0.116×10^{-5}
$\mu g\ (\mu m^3\ chloroplast)^{-1}$	1.763×10^{-4}	0.300×10^{-4}	1.093×10^{-4}	0.043×10^{-4}
Photosynthesis (O_2 evolved)				
$\mu mol\ cell^{-1} \cdot min^{-1}$	2.131×10^{-10}	0.133×10^{-10}	1.905×10^{-10}	0.074×10^{-10}
$\mu mol\ (\mu m^3\ protoplast)^{-1} \cdot min^{-1}$	3.311×10^{-13}	0.197×10^{-13}	3.141×10^{-13}	0.138×10^{-13}
$\mu mol\ (\mu m^3\ chloroplast)^{-1} \cdot min^{-1}$	1.786×10^{-12}	0.400×10^{-12}	1.166×10^{-12}	0.051×10^{-12}
Respiration (O_2 consumed)				
$\mu mol\ cell^{-1} \cdot min^{-1}$	1.276×10^{-10}	0.061×10^{-10}	1.613×10^{-10}	0.218×10^{-10}
$\mu mol\ (\mu m^3\ protoplast)^{-1} \cdot min^{-1}$	1.984×10^{-13}	0.089×10^{-13}	2.658×10^{-13}	0.345×10^{-13}
$\mu mol\ (\mu m^3\ mitochondria)^{-1} \cdot min^{-1}$	3.905×10^{-12}	0.176×10^{-12}	5.383×10^{-12}	0.699×10^{-12}
Carbon				
$mg \cdot cell^{-1}$	1.398×10^{-7}	0.002×10^{-7}	1.310×10^{-7}	0.002×10^{-7}
$mg\ (\mu m^3\ protoplast)^{-1}$	2.174×10^{-10}	0.003×10^{-10}	2.161×10^{-10}	0.015×10^{-10}
Nitrogen				
$mg \cdot cell^{-1}$	2.904×10^{-8}	0.077×10^{-8}	2.377×10^{-8}	0.022×10^{-8}
$mg\ (\mu m^3\ protoplast)^{-1}$	4.514×10^{-11}	0.105×10^{-11}	3.919×10^{-11}	0.015×10^{-11}

Amphidinium carterae chromosomes were altered ultrastructurally with 10^{-3} M selenate. The chromosomes were less osmiophilic and the perichromatic granules more densely stained than in the control cells. Selenium increased the amount of chromosomal aberrations (breaks and gaps) in *Allium sativum* root tips (Mukherjee and Sharma 1988) and caused DNA strand breakage in fibroblasts and hepactocytes (Snyder 1988; Garberg et al. 1988), while also inhibiting both RNA and DNA polymerase activities (Frenkel et al. 1987). The last effect is especially hazardous, since it would decrease the ability to repair damaged genetic material. However, it is unlikely that the chromosomal alterations observed in *Amphidinium carterae* were deleterious, since the cells were able to successfully adapt to 10^{-3} M selenate (Wong and Oliveira 1991). The function of perichromatic granules in dinoflagellates is unknown (Spector 1984). The significance of their change is also unknown.

In conclusion, the effects observed on the ultrastructure, volume of mitochondria, and respiration rate, together with the alterations observed in the chloroplasts and reduction in the amount of chlorophyll *a* and photosynthesis, suggest that energy-transducing systems were altered by selenium toxicity. In the long run, this leads to decreases in the amount of storage products which is indicative of severe shortages in energy. However, despite these severe effects, the microalgae tested were able to adapt to high selenium concentrations (Wong and Oliveira 1991). Although adaptation mechanisms are far from understood in these and other microorganisms, the potential for adaptation to high selenium concentrations must be considered if meaningful conclusions are to be drawn from studies on selenium toxicity.

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Temperature Acclimation of Xenobiotic Metabolizing Enzymes in Cultured Hepatocytes and Whole Liver of the Gulf Toadfish, *Opsanus beta*

Christopher J. Kennedy, Kenneth A. Gill, and Patrick J. Walsh[1]

Divison of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149-1098, USA

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Changes in cytochrome P-450 and microsomal and soluble protein content and the activities of several microsomal and cytosolic xenobiotic metabolizing enzymes were examined in the whole livers of gulf toadfish, *Opsanus beta*, following acclimation to 18 or 28°C and in isolated hepatocytes (prepared from 18 or 28°C acclimated gulf toadfish) which were maintained in primary culture for 29 d on L-15 media. Cells isolated from both acclimation groups were cultured at 18 and 28°C. In whole livers, significant differences were found in the activities of sulfotransferase (which showed perfect compensation) and UDP-glucuronosyltransferase (which showed "inverse compensation") between fish acclimated to 18 and 28°C, but several other trends were obscured by interindividual variation. However, in hepatocyte cultures, temperature adaptations were seen in cytochrome P-450 content and in the activities of the microsomal enzymes aryl hydrocarbon hydroxylase, epoxide hydrolase, and UDP-glucuronosyltransferase. Glutathione-S-transferase activity appeared to be rather temperature insensitive in both whole livers and in cultured hepatocytes. Incomplete temperature acclimation for all enzymes was observed in cultured cells and this may be due to systemic factors which were not present in cell culture media.

Les changements de la concentration du cytochrome P-450 et des protéines microsomiques et des protéines solubles et l'activité de plusieurs enzymes microsomiques et cytosoliques qui métabolisent les composés xénobiotiques ont été examinés dans des foies entiers de crapaud de mer, *Opsanus beta*, après acclimatation à 18 ou 28°C et dans des hépatocytes isolés (préparés à partir de crapauds de mer acclimatés à 18 ou 28°C) qui ont été maintenus en culture primaire pendant 29 jours dans du milieu L-15. Les cellules isolées des deux groupes acclimatés ont été cultivées à 18 et 28°C. Dans les foies entiers, on a observé des différences significatives dans l'activité de la sulfotransférase (compensation directe) et de l'UDP-glucuronosyltransférase (« compensation inverse ») entre les poissons acclimatés à 18 et 28°C, mais plusieurs autres tendances ont été masquées par les variations entre individus. Cependant, dans les cultures d'hépatocytes, on observe des adaptations à la température dans la concentration de cytochrome P-450 et dans l'activité des enzymes microsomiques que sont l'aryl-hydrocarbure-hydroxylase, l'époxyde-hydrolase et l'UDP-glucuronosyltransférase. L'activité de la glutathion-S-transférase semble être relativement peu sensible à la température aussi bien que les foies entiers que dans les cultures d'hépatocytes. On a observé une acclimatation incomplète à la température pour toutes les enzymes dans les cellules cultivées, ce qui peut être dû à des facteurs systémiques qui n'étaient pas présents dans les milieux de culture.

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Interest in fish species (with variable body temperatures) as alternate model systems in toxicological and cancer research (Hoover 1984; Power 1989) and the proposed use of biotransformation enzyme levels as tools in biological monitoring (Payne et al. 1987) make it important to study the effects of environmental variables such as temperature on xenobiotic metabolism. Metabolic adaptation to environmental temperature fluctuations in ectotherms may be partially attributed to the alteration of enzyme quantity or isozymes present in a tissue. For example, when temperature changes persist, in many energy-transforming metabolic pathways, compensatory adjustments of enzyme activities are made to offset the direct effects of temperature (Hazel and Prosser 1974; Hochachka and Somero 1984; Koban 1986). Temperature has also been shown to affect hepatic enzymes involved in the metabolism of xenobiotics, in particular ithe cytochrome P-450 dependent mixed-function oxidase (MFO) system. The MFO system is responsible for the oxidation of many endogenous compounds (e.g. steroids, vitamins, and fatty acids), but it also catalyzes the first step (i.e. Phase I) in the detoxification of exogenous compounds (e.g. pesticides and polycyclic aromatic hydrocarbons). However, in this process some compounds may actually be metabolically "activated" to more toxic forms by MFOs. For example, the reactive Phase I metabolite BaP-7,8-diol-9,10-epoxide is believed to be the ultimate carcinogen of benzo[*a*]pyrene. Thus, alterations in metabolic capacity by variables such as temperature may have substantial effects on tissue levels of toxicants, metabolite patterns, and xenobiotic half-life in an organism, all of which may affect the severity and duration of a toxic response (Buhler and Williams 1989). The

[1]Author to whom correspondence should be addressed.

effects of temperature on these enzymes are varied and the mechanisms of the effects remain unclear. Metabolic compensation to temperature (i.e. when fish are acclimated to lower temperatures, enzyme activities are increased to offset the acute effects of temperature (Hazel and Prossser 1974)) has been shown to occur for MFO activities in several fish species (Stegeman 1979; Egaas and Varanasi 1982; Koivusaari 1983; Ankley et al. 1985; Lindstrom-Seppa 1985). Conversely, several studies have shown that levels of other Phase I activities exhibit "inverse" or "paradoxical" compensation (Hazel and Prosser 1974), i.e. activity increases with increasing acclimation temperature (Karr et al. 1985; Jimenez et al. 1988; Jimenez and Burtis 1989). Fewer data are available for Phase II (conjugating) enzyme systems, but it has been reported that UDP-glucuronosyltransferase exhibits inverse compensation (Koivusaari 1983; Andersson and Koivusaari 1985).

In achieving these new levels of enzyme activity, a range of factors could be regulating the response, from, for example, simple direct effects of temperature on rates of synthesis/degradation to more complex hormonally mediated responses. However, few studies have attempted to localize the regulatory mechanisms responsible for these enzyme adjustments in fish, and cultured fish hepatocytes (Moon et al. 1985) appear to be an excellent system with which to study this phenomenon. In a study of the effects of temperature on energy-transforming enzymes in cultured catfish hepatocytes, Koban (1986) found that regulation of much of the response to temperature rested with the cell itself, independent of organismal level responses. To our knowledge this approach has not been used for enzymes of xenobiotic metabolism. Given the especially high number of enzymes exhibiting inverse compensation, this approach could yield interesting insights into their regulation. For these reasons, in this study, we compared the effects of temperature acclimation on several Phase I and Phase II enzymes in freshly isolated livers and in cultured hepatocytes of the gulf toadfish, *Opsanus beta*. Prior studies have established temperature as an important modifier of xenobiotic uptake, metabolism, and disposition in this species (Kennedy et al. 1989a, 1989b; Gill and Walsh 1990).

Materials and Methods

Experimental Organism and Acclimation Regimen

Mature specimens of the gulf toadfish, weighing 17–346 g, were collected from Biscayne Bay, FL, by roller trawl by a local fisherman in August and September 1989. Fish were acclimated to either 18 or 28°C ± 1.0°C in flow-through seawater aquaria (salinity 33‰) and a natural photoperiod for 4 wk prior to sacrifice for the determination of hepatic enzyme activities or isolation of hepatocytes for culture. Fish were fed frozen squid twice a week until 1 wk before an experiment. Survival through treatment was 95%; no diseases were obvious in experimental fish.

Isolation of Hepatocytes

Hepatocytes were isolated from toadfish, as in Walsh (1987), with modifications to insure sterility. Following anesthesia with 0.5 g MS 222·L^{-1}, fish were swabbed with 70% ethanol, and surgery was performed in a sterile field. Livers were initially perfused for 25 min with a sterile Ca^{2+}- and Mg^{2+}-free Hanks-salts solution containing collagenase (95 units·mL^{-1}; Sigma C5138). The hepatocytes were then gently disaggregated by filtration through nylon mesh, concentrated by low-speed centrifugation, and resuspended in L-15 medium. Centrifugation and resuspension were repeated two more times.

Hepatocyte Cultures

Cells were seeded at a starting density of 0.6 × 10^6 to 1.2 × 10^6 cells·mL^{-1} (7 mL total) in Primaria T-25 flasks (Falcon). L-15 medium (GIBCO, Grand Island, NY) (adjusted to pH 7.6) contained added antibiotics (50 μg gentamycin·mL^{-1} and 2.5 μg fungizone·mL^{-1}) but no serum supplements. Cells were isolated from fish acclimated to 18 or 28°C and cultured either at the original acclimation temperature (controls) or at the opposite experimental temperature. Cells were fed by total replacement of medium every 2–3 d. The hepatocytes were cultured under atmospheric air for up to 29 d and medium C_{CO_2} was approximately 1.0 mmol·L^{-1}.

Enzyme Activities

Livers from temperature-acclimated gulf toadfish were prepared as follows. Fish were anesthetized with 0.5 g MS 222·L^{-1}. The liver was removed and homogenized at 4°C with a Potter-Elvejhem glass-teflon homogenizer in 4 volumes of ice-cold homogenization buffer (0.15 M KCl, 0.02 M HEPES, pH 7.4). An aliquot was then removed and placed into a 2-mL plastic tube (Sarstedt, Princeton, NJ) and assayed for DNA content according to Erwin et al. (1981) using calf thymus DNA (Sigma D1501) as a standard. After the addition of 750 μL of 10% TCA, the tube was incubated at 4°C for 4 h and centrifuged at 13 000 × *g* for 1 min on a Fisher model 235B microcentrifuge. The supernatant was discarded and 500 μL of 5% TCA was added and the pellet gently broken up. The solution was then hydrolyzed at 90°C for 30 min followed by cooling at 4°C. The solution was centrifuged again at 13 000 × *g* and the pellet discarded. To the supernatant was added 100 μL of 3,5-diaminobenzoic acid dihydrochloride (200 mg·mL^{-1}) followed by incubation for 1 h at 60°C. After incubation, 2 mL of 1 N HCl was added and the fluorescence read on a Perkin Elmer LS-3B (Norwalk, CT) spectrofluorometer (E_x = 380 nm, E_m = 520 nm).

Cultured cells were harvested at various times following initiation of culture by replacing the L-15 medium with Hanks balanced salts solution containing collagenase (95 units·mL^{-1}) and gently agitating on a reciprocating shaker for 30 min, followed by gentle removal of any remaining adhering cells from the flask bottom with a teflon scraper. Cells were collected by centrifugation at 500 × *g* and resuspended in a small volume of ice-cold homogenization buffer. An aliquot was removed for DNA quantitation as above. Cells were counted on a hemocytometer and then disrupted by sonication.

Homogenates from whole livers and cultured cells were centrifuged at 10 000 *g* for 20 min in a Sorvall RC5B superspeed centrifuge and the pellet discarded. The resulting supernatant was centrifuged at 100 000 × *g* for 60 min at 4°C on a model L7 Beckman ultracentrifuge. The 100 000 × *g* supernatant was removed and the surface of the microsomal pellet was rinsed gently with 1 mL of the homogenization buffer. Microsomes were resuspended in homogenization buffer plus 20% glycerol (v/v). Assays with microsomes and supernatant were performed immediately.

Microsomal and supernatant protein concentrations were measured by the method of Lowry et al. (1951), with bovine serum albumin as a standard.

Cytchrome P-450 content was determined by the method of Omura and Sato (1964), which measured the CO-difference spectra of dithionite-reduced microsomes.

Aryl hydrocarbon hydroxylase (AHH) activity was determined by the modified radiometric procedure of Van Cantford et al. (1977), as described by Collier et al. (1986). A mixture of 450 μL of 50 mM Tris/HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM NADPH, and 37.5 μL of microsomal suspension was preincubated for 5 min at the assay temperature. The addition of 12.5 μL of acetone containing 40 nmol (2 μCi = 74 kBq) of [^{14}C]benzo[*a*]pyrene (BaP, Amersham, Arlington Hts., IL) started the reaction. Incubations were for 15 min and the reaction was stopped by the addition of 1 mL of 0.15 M KOH in 85:15 (v/v) DMSO:H_2O. The reaction mixture was extracted three times with 5 mL of hexanes to remove the unreacted BaP. The aqueous layer was neutralized with 0.5 mL of 0.1 N HCl and 0.5 mL was removed and counted by LSC. In control flasks, KOH:DMSO was added before BaP.

UDP-glucuronosyltransferase (UDPGT) activity was measured by a modified spectrophotometric method of Hellman and Touster (1962). The reaction mixture, in a total volume of 0.6 mL, contained 60 μL of 1.7 mM *p*-nitrophenol in 0.5 M KH_2PO_4/K_2HPO_4, pH 7.4, 100 μL of 0.1 mM UDP-glucuronic acid, 50 μL of microsomal suspension, 140 μL of H_2O, and 250 μL of 0.1% Triton X-100 in 0.5 M KH_2PO_4/K_2HPO_4. The reaction was allowed to continue for 30 min and was stopped by the addition of 2.4 mL of 0.1 N TCA. The mixture was centrifuged at 2000 × *g* for 10 min and 60 μL of 10 N KOH was added to the supernatant. Control vials contained no UDP-glucuronic acid. The absorbance was measured at 401 nm.

Epoxide hydrolase (EH) activity was assayed by the modified radiometric procedure of DePierre et al. (1978) according to Collier et al. (1986). Reaction vials contained 75 μL of 0.5 M Tris/HCl, pH 7.5, and 25 μL of microsomal suspension. A reaction was started by the addition of 40 μL of acetone containing 0.58 μmol (3.6 μCi = 132.2 kBq) of [^{3}H]styrene oxide (Amersham) and was continued for 30 min. The reaction was stopped by the addition of 5 mL of hexanes. Water (1.9 mL) was added to the vial and the organic phase discarded. The aqueous layer was extracted three times with 5 mL of hexanes to remove any remaining substrate. A 1-mL aliquot of the aqueous phase was removed and counted by LSC. In the control vial, hexane was added before [^{3}H]styrene oxide.

Glutathione-S-transferase (GST) in the supernatant was measured spectrophotometrically as described by Habig et al. (1974). The reaction mixture consisted of 1.95 mL of 50 mM HEPES, pH 7.5, 40 μL of 1-chloro-2,4-dinitrobenzene, and 25 μL of 100 000 × *g* supernatant. The reaction was started by adding 25 μL of 50 mM reduced glutathione in 50 mM HEPES. The change in absorbance at 340 nm was monitored for 1–2 min. Controls were performed both without supernatant or glutathione in reaction mixture.

Sulfotransferase (SFT) in the supernatant was assayed using the modified radiometric procedure of Nemoto et al. (1977). The reaction mixture consisted of 0.86 mL of 50 mM Tris/HCl, pH 7.4, 3 mM $MgCl_2$, 2 mM $MgSO_4$, and 5 mM ATP and 20 μL of 0.15 KCl, 0.02 M HEPES, pH 7.4, and 20% glycerol (v/v) containing 50 units of ATP sulfurylase (Sigma A5761). This mixture was incubated for 30 min at room temperature to ensure the formation of 3-phosphoadenosine 5′-phosphosulfate. The assay was started by the addition of 100 μL of 100 000 × *g* supernatant followed by 0.19 nmol (20 μCi = 740 kBq) of [^{3}H]styrene oxide in 20 μL of acetone. The reaction was terminated at 30 min by the addition of 5 mL of hexanes. The mixture was extracted three times with hexanes to remove unreacted substrate. The remaining aqueous layer was adjusted to pH 7.0 and incubated for 24 h at 37°C with 20 U sulfatase (Sigma S1629). The aqueous layer was extracted until no further radioactivity was detected in the extracts, and an aliquot of the combined organic layers, which contained the styrene oxide residues of sulfate conjugates, was counted for total radioactivity by LSC. In the control reaction, hexane was added before the supernatant.

All enzyme activities measured were linear with time during the incubation period. A LKB Biochrom Ultrospec 4050 spectrophotometer was used for spectral measurements. Radioactivity was counted on a Tracor Beta Trac liquid scintillation counter. ACS (Amersham) was used as scintillation liquid in LSC. All biochemicals were purchased from Sigma (St. Louis, MO).

Calculations and Statistics

All values are reported as means ± SE. Enzyme activity in whole livers is expressed on a per gram wet weight basis and for cultured cells as a percentage of initial control levels in 18°C acclimated cells. All data were compared using a paired Student *t*-test (Zar 1974) to test for significant differences between temperature groups at the $p < 0.05$ level.

Results

Enzyme Activities in Whole Livers

There was no significant difference in the hepatosomatic index (liver wt./body wt. × 100) between fish acclimated to either 18 or 28°C (Table 1). Table 1 also shows that there was no significant difference in DNA content, microsomal protein, soluble protein, or cytochrome P-450 levels between fresh whole livers from fish acclimated to high or low temperature.

The activities of several microsomal (AHH, UDPGT, and EH) and cytosolic (GST and SFT) biotransformation enzymes are shown in Table 2. Although a wide body weight range was used in these experiments, there did not appear to be any effect of weight on the enzyme activities examined. All enzymes, except AHH, showed a significant increase in activity with increasing incubation temperature. The effects of acclimation temperature on enzyme activities from fresh livers at each incubation temperature are also shown in Table 2. SFT activity was significantly lower in livers from 28°C acclimated fish than in livers from fish acclimated to 18°C at all incubation temperatures. The acclimatory Q_{10} (ratio of the rate at 18°C to the rate at 28°C) was approximately 2.0, indicating compensation. UDPGT activity was elevated in livers from 28°C acclimated fish but the difference was only significant at an incubation temperature of 18°C. The acclimatory Q_{10} was approximately 0.5, indicating inverse compensation. AHH, EH, and GST showed no significant changes with acclimation temperature.

Hepatocyte Viability

As in prior studies (Walsh 1987), freshly isolated gulf toadfish hepatocytes consistently showed greater than 97% (typically >99%) viability as judged by trypan blue exclusion. Cells in culture harvested up to 29 d always showed greater than 95% trypan blue exclusion. Gulf toadfish hepatocyte preparations

TABLE 1. Body weight and hepatosomatic index as well as DNA, cytochrome P-450, microsomal, and soluble protein content in freshly isolated livers of gulf toadfish acclimated to either 18 or 28°C. Values are the means ± SE of six fish. There were no significant differences between acclimation groups in any of the parameters at $p < 0.05$. Protein contents in cultured cells on day 0 from fish acclimated to 18°C are given for comparison with levels in whole liver.

	Acclimation temperature (°C)		
	18	28	Cultured cells
Body weight (range, g)	26–308	17–128	
Hepatosomatic index	0.96 ± 0.08	0.95 ± 0.07	
DNA ($\mu g \cdot g^{-1}$)	2953 ± 220	2337 ± 279	
Soluble protein ($mg \cdot g^{-1}$)	94.6 ± 8.7	113.1 ± 12.6	82.5 ± 14.2
Microsomal protein ($mg \cdot g^{-1}$)	9.5 ± 3.3	12.5 ± 4.5	7.25 ± 1.10
Cytochrome P-450			
$nmol \cdot g^{-1}$	1.68 ± 0.20	1.21 ± 0.17	2.18 ± 0.78
nmol·mg protein^{-1}	0.18 ± 0.03	0.10 ± 0.04	0.30 ± 0.11

TABLE 2. Aryl hydrocarbon hydroxylase (AHH), epoxide hydratase (EH), glutathione-S-transferase (GST), UDP-glucuronosyltransferase (UDPGT), and sulfotransferase (SFT) activities at three incubation temperatures from whole livers of 18 or 28°C acclimated gulf toadfish. Values are means ± SE of six fish. *Significant difference from 18°C acclimated fish; **significant difference from 18°C incubation; significance level is $p < 0.05$. Results are similar when activities are based on a per milligram of protein, per gram of liver, cytochrome P-450, or DNA content basis. Enzyme activities are given for cell cultures from 18°C acclimated fish on day 0 for comparison with values from whole liver.

Enzyme	Acclimation temperature (°C)	Incubation temperature (°C)			Cultured cells
		18	23	28	
AHH	18	0.276 ± 0.102	0.335 ± 0.114	0.396 ± 0.115	0.399 ± 0.104
($nmol \cdot min^{-1} g^{-1}$)	28	0.184 ± 0.065	0.283 ± 0.087	0.303 ± 0.085	
EH	18	7.13 ± 1.32	8.67 ± 1.42	10.45 ± 1.67**	4.24 ± 0.72
($nmol \cdot min^{-1} \cdot g^{-1}$)	28	9.05 ± 0.89	10.16 ± 1.24	12.90 ± 1.09**	
GST	18	0.041 ± 0.002	0.046 ± 0.003	0.062 ± 0.003**	0.024 ± 0.004
($mmol \cdot min^{-1} \cdot g^{-1}$)	28	0.040 ± 0.003	0.044 ± 0.003	0.055 ± 0.004**	
UDPGT	18	1.21 ± 0.17	1.73 ± 0.28	2.47 ± 0.38**	1.58 ± 0.16
($nmol \cdot min^{-1} \cdot g^{-1}$)	28	2.27 ± 0.41*	3.10 ± 0.69	5.58 ± 1.24**	
SFT	18	4.92 ± 0.33	6.01 ± 0.42	13.24 ± 1.20**	2.42 ± 0.30
($nmol \cdot min^{-1} \cdot g^{-1}$)	28	2.33 ± 0.52*	3.90 ± 0.54*	7.11 ± 0.40*,**	

were almost exclusively parenchymal cells which is similar to other fish hepatocyte preparations (Hinton et al. 1985). There was minimal contamination by pancreatic cells because gulf toadfish have Brockman bodies located separately on the hepatic portal veins, and other cell types (e.g. fibroblasts) were not observed. Under phase-contrast microscopy, cells which had been in culture for 1 wk or more were flattened, and cell–cell contact and a typical cordlike structure (Koban 1986) were evident.

Enzyme Activities in Hepatocytes

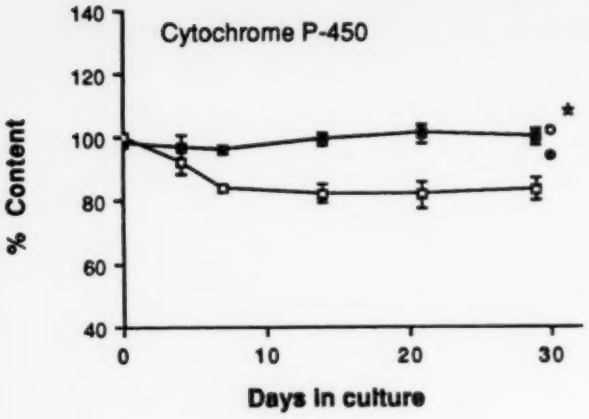
Enzyme activities, cytochrome P-450, and protein contents of cultured hepatocytes from each acclimation group were relatively variable, and in order to more readily compare the results of each experiment, the data were expressed as percent changes from "100% activity" at day 0 based on the average activity of 18°C acclimated cells. No differences in trends or statistics were evident when enzyme activities were first expressed on a cell number, DNA, or protein content basis.

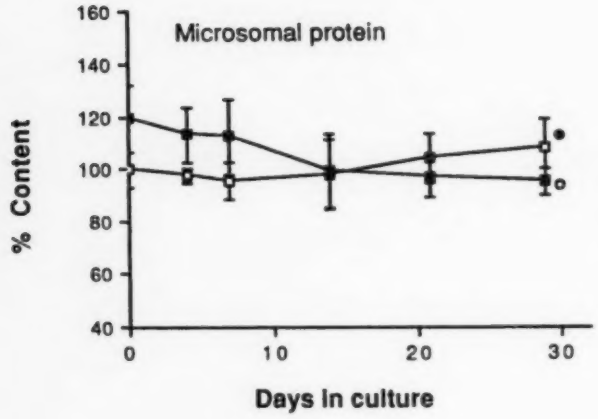
In control cultures, cells were incubated at their acclimation temperature and were sampled at day 29 in order to assess the effects of the culture conditions on enzyme activities or protein contents apart from temperature effects. In all cases, enzyme activities and protein contents in control cells remained relatively constant for the duration of the experiment which may be taken as an additional indication of continued hepatocyte viability (Fig. 1–3). As well, when activities or protein contents were calculated on a per gram wet weight basis, levels were similar to those obtained in whole livers (Tables 1 and 2).

The time course of changes in cytochrome P-450, microsomal protein, or soluble protein in the cultured cells is depicted in Fig. 1. Cells isolated from 18°C acclimated fish and cultured at 28°C decreased the levels of cytochrome P-450 significantly, but the reverse effect was not seen. The amount of microsomal and soluble protein in cells did not change significantly during acclimation to a new temperature.

The changes in microsomal enzyme activities (AHH, EH, and UDPGT) are given in Fig. 2. Both AHH and EH activities increased during incubation in colder temperatures, while significantly decreasing in cells which were incubated at higher temperatures. UDPGT activity showed a reverse trend to that of AHH and EH activities, i.e. increasing incubation temperature increased UDPGT activity and decreased activity was seen in cells incubated to the lower temperature. Values for enzyme activity in cells incubated at their original acclimation temperatures were similar to freshly isolated hepatocytes, indicating that AHH, EH, and UDPGT activities were not adversely affected by the culture conditions.

The activities of the cytosolic enzymes GST and SFT are shown in Fig. 3. Changes in temperature and culture conditions did not appear to significantly alter the activities of GST or SFT





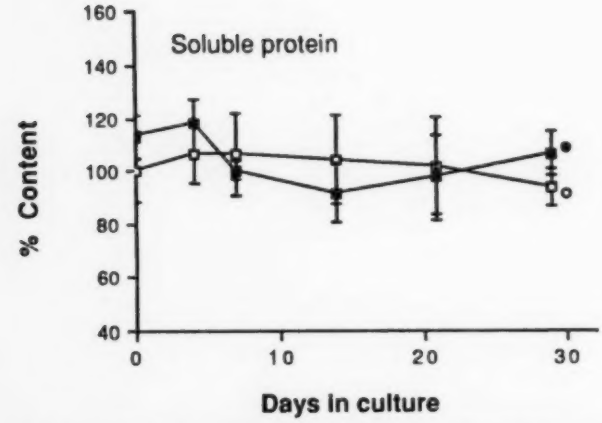
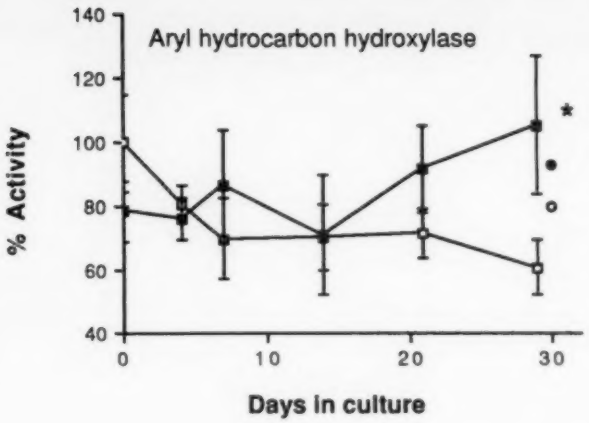
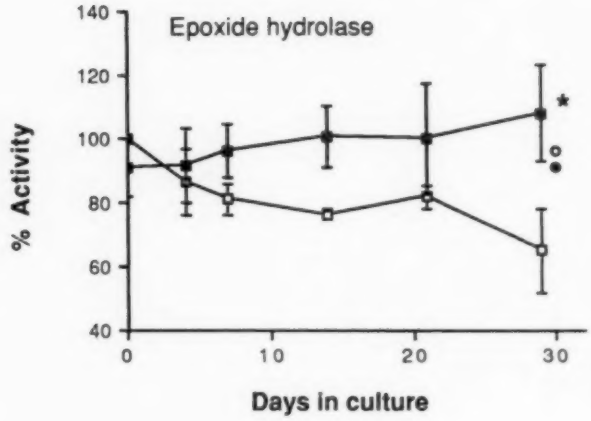


FIG. 1. Effect of culture temperature on cytochrome P-450 and microsomal and soluble protein content in cultured hepatocytes. Hepatocytes from 18°C acclimated fish were cultured at 28°C (□) and cells from 28°C acclimated fish were cultured at 18°C (■). Control flasks from 18°C (○) or 28°C (●) acclimated fish were cultured at their acclimation temperature. Cytochrome P-450 or protein levels (mg·million cells^{-1}) are expressed as percent changes from day 0 which was normalized to 100% for the average value of 18°C acclimated cells. *Significant difference at the $p < 0.05$ level at day 29.





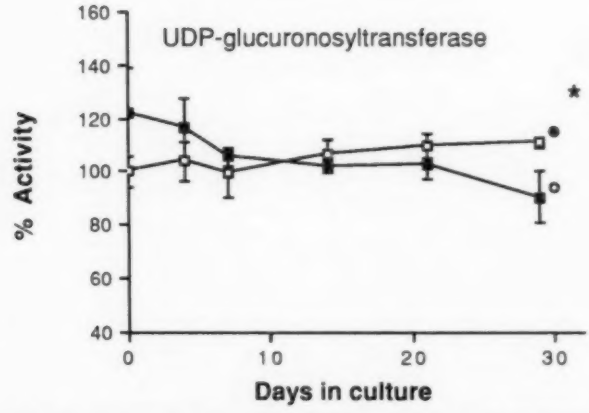


FIG. 2. Effect of culture temperature on the activities of the microsomal enzymes aryl hydrocarbon hydroxylase, epoxide hydratase, and UDP-glucuronosyltransferase in cultured hepatocytes. Hepatocytes from 18°C acclimated fish were cultured at 28°C (□) and cells from 28°C acclimated fish were cultured at 18°C (■). Control flasks from 18°C (○) or 28° (●) acclimated fish were cultured at their acclimation temperature. Enzyme activities (nmol·min^{-1}·million cells^{-1}) are expressed as percent changes from day 0 which was normalized to 100% for the average value of 18°C acclimated cells. *Significant difference at the $p < 0.05$ level at day 29.

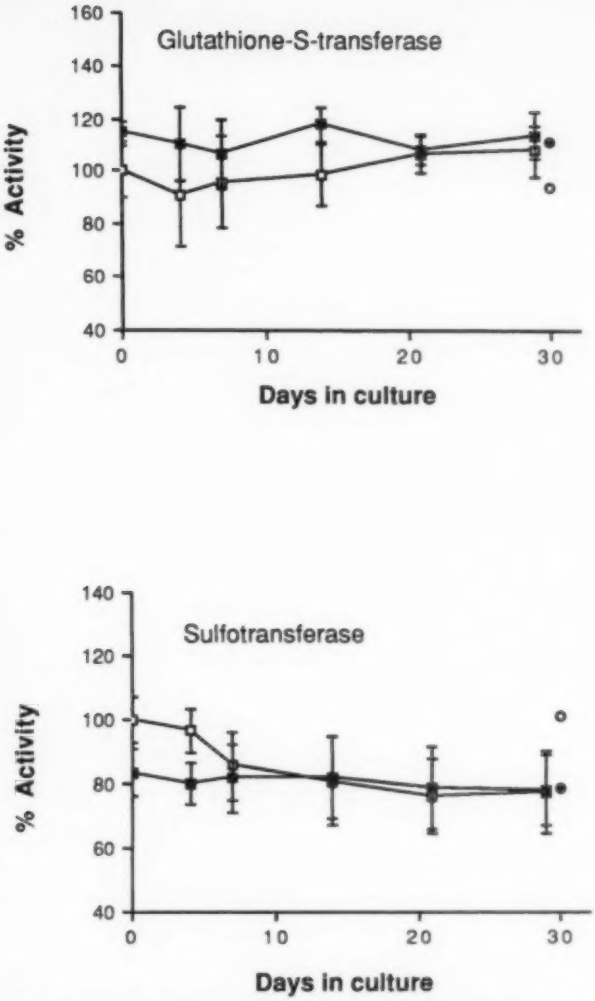


FIG. 3. Effect of culture temperature on the activities of the cytosolic enzymes glutathione-S-transferase and sulfotransferase in cultured hepatocytes. Hepatocytes from 18°C acclimated fish were cultured at 28°C (□) and cells from 28°C acclimated fish were cultured at 18°C (■). Control flasks from 18°C (○) or 28°C (●) acclimated fish were cultured at their acclimation temperature. Enzyme activities (mmol·min^{-1}·million cells^{-1} for GST, nmol·min^{-1}·million cells^{-1} for SFT) are expressed as percent changes from day 0 which was normalized to 100% for the average value of 18°C acclimated cells. *Significant difference at the $p < 0.05$ level at day 29.

in the cells. The activities of both GST and SFT in control flasks remained constant to day 29 in culture.

Discussion

In the present study, we acclimated gulf toadfish to 18 and 28°C for several weeks and examined hepatosomatic index, microsomal and soluble protein content, and cytochrome P-450 content, as well as the activities of several Phase I (AHH and EH) and Phase II (GST, SFT, and UDPGT) enzymes, under saturating and optimal conditions at three assay temperatures. Our results indicated that there was no significant difference in liver weight to body weight ratios between the groups of fish acclimated to 18 or 28°C. The results of other studies are mixed and have shown that in some cases, fish acclimated to colder temperatures have increased hepatosomatic indices (Kent et al. 1988; Walsh et al. 1983) whereas others have noted smaller indices when fish are acclimated to lower temperatures (Stegeman 1979). Hypertrophy of the liver may not be seen in gulf toadfish, a subtropical species, as is seen in more temperate fish species (Kent et al. 1988; Walsh et al. 1983) because of the smaller seasonal shifts in temperature experienced by gulf toadfish. However, others have indicated that hepatosomatic index is due to a complex of environmental and physiological factors such as water temperature, food availability, and reproductive state (Koivusaari 1983).

No significant differences in cytochrome P-450 or microsomal or soluble protein content were found in the livers of the two groups of gulf toadfish, but a trend towards increased cytochrome P-450 at a lower temperature was noted (Table 1). Koivusaarai (1983) also found that microsomal protein content did not change with season in the liver of rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*); however, cytochrome P-450 content was significantly lower in fish during early spring and he postulated that this was mainly due to factors associated with the reproductive state of the fish.

Three enzymes (AHH, EH, and GST) displayed a relatively low sensitivity to acute temperature changes, and consequently only minor acclimatory adjustments are made. However, the Phase II enzymes UDPGT and SFT showed a marked temperature sensitivity and acclimatory adjustment. SFT exhibits nearly perfect compensation, but UDPGT exhibits "inverse compensation" (Hazel and Prosser 1974). These results suggest that flux through UDPGT is deemphasized at colder temperatures. Previous in vivo studies have shown that there was no significant difference in the proportion of BaP–glucuronide conjugates in the bile of gulf toadfish which were exposed to BaP in water at acclimation temperatures or following an acute temperature change (Kennedy et al. 1989b), which indicates that the change in UDPGT activities may be related to other functions of the enzyme. However, our choice of synthetic substrate (*p*-nitrophenol) makes direct comparisons of changes in activities with in vivo BaP metabolic rates difficult.

The collection area for gulf toadfish has relatively low levels of pollutants (Corcoran et al. 1983), and therefore, it is assumed that fish used in this study had basal titres of xenobiotic transforming enzymes at the beginning of the experiments. Even so, some enzyme activities showed a relatively high variability within an acclimation group, indicating that other factors may play a role in modifying enzyme activities. This wide variability is also observed in other "wild fish populations" from both polluted and "non-polluted" sites, which is the major difficulty in using such enzymes as a biomonitoring tool for indices of aquatic pollution (Jimenez and Burtis 1989). This variability may mask any effects of temperature acclimation and can be reduced if measurements of xenobiotic transforming enzymes are made on cells obtained from the same fish acclimated to different temperatures in a paired design. In this regard, hepatocyte cultures were used to determine if adjustments of xenobiotic transforming enzymes occur, and if so, whether these adjustments are a direct result of thermal modulation at the cellular level and independent of systemic regulation.

As in freshly isolated livers, there appeared to be no significant differences or changes in the content of microsomal or soluble protein in the freshly isolated hepatocytes. Significant

differences in the content of cytochrome P-450 were not seen in freshly isolated livers; however, in cultured cells the percent change in cytochrome P-450 levels in cells acclimating to cold temperatures was significantly higher than in cells acclimating to warmer temperatures.

Although significant differences in both microsomal Phase I enzymes AHH and EH were not seen in freshly isolated livers, there were significant differences seen in cultured cells. Acclimation to colder temperatures increased the activities of both of these enzymes, and acclimation to warmer temperatures resulted in a decreased enzyme activity. Increased activity of AHH in response to cold acclimation has been reported in several fish species (Varanasi 1989), and Egaas and Varanasi (1982) have reported that a significantly larger hepatic EH activity is found in rainbow trout maintained at low temperatures. There are several factors which may be responsible for the increased activity of these microsomal enzymes in response to cold acclimation: (1) quantitative changes in the amount of enzyme present (Sidell 1983), (2) the production of different cytochrome P-450 isozymes with different catalytic capability, which may be triggered by cold acclimation, and (3) alterations in membrane lipid composition, due to changing temperature, which may modify enzyme activity. For example, Wills (1980) reported a higher rate of BaP oxidation associated with a higher proportion of polyunsaturated fatty acids in microsomal membranes, a phenomenon seen in membranes of cold-acclimated fish (Hazel 1979). Our results indicate that for AHH, part of the response may be explained by changes in the amounts of cytochrome P-450, and these changes can be initiated at least in part by direct effects of temperature on the cells.

UDPGT activity showed "inverse compensation' in both freshly isolated livers and in hepatocyte cultures, indicating that control of this response is initiated directly by temperature effects on the cell. This decrease in activity associated with decreasing water temperature is also seen in rainbow trout (Koivusaari 1983), and it has been suggested that the regulation of hepatic glucuronidation by ambient temperature is important in the control of plasma steriod levels during the reproductive season (Andersson and Koivusaari 1985). Low water temperatures and the concomitant low activity of glucuronidation enzyme activities may enable the fish to maintain the high levels of steroid hormones necessary for reproductive development (Kime and Saksema 1980; Scott and Sumpter 1983; Andersson and Koivusaari 1985). Thus this enzyme would appear to play a smaller role in xenobiotic detoxification in some fish species than in mammals, and our results in this study and previously (Kennedy et al. 1989b) support this view.

Although SFT showed a marked temperature compensation in activity in fresh livers, the results with cells in culture were not clear. There appeared to be an increased SFT activity in cold-acclimated cells at the beginning of the experiment; however, after 1 wk the activity in both groups of cells was similar. The degree of compensation may not be complete in cultured hepatocytes due to the absence of specific macromolecular supplements in the culture medium. Koban (1986) also found incomplete temperature compensation in several mitochondrial and cytosolic enzymes in cultured catfish hepatocytes and attributed this to a missing systemic factor such as hormones. Thus the adjustments of SFT activity appear to rely more directly on a systemic level response than other enzymes examined.

As in fresh livers, the activity of GST in cultured hepatocytes appeared to be relatively temperature insensitive. Levels of GST were similar in cells from both cold- and warm-acclimated fish, and the change in percent activity over the 29-d culture period was minimal. GST appears to be the primary Phase II detoxification enzyme in gulf toadfish (Kennedy et al. 1989a, 1989b; Gill and Walsh 1990), and we have speculated that "positive thermal modulation," i.e. an increase in K_m with temperature (Hochachka and Somero 1984), is responsible for its apparent temperature insensitivity.

In conclusion, this study demonstrates that cultured hepatocytes are a useful model system in the study of factors which affect and regulate xenobiotic metabolism in fish. Trends in the changing activities of several microsomal and cytosolic xenobiotic metabolizing enzymes (cytochrome P-450, AHH, and EH) which were not seen when measured in whole livers were unmasked when variation between individuals was reduced in culture. Partial temperature adaptation was seen for most of the enzymes studied; however, it appears that complete acclimation to a new temperature is reliant upon a higher organizational level than the cell for some enzymes.

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Are Chloride Cells Involved in Low pH Tolerance and Sensitivity? The Mayfly Possibility

Michael Berrill, Graeme Taylor, and Helene Savard

Watershed Ecosystems, Trent University, Peterborough, Ont. K9J 7B8, Canada

Berrill, M., G. Taylor, and H. Savard. 1991. Are chloride cells involved in low pH tolerance and sensitivity? The mayfly possibility. Can. J. Fish. Aquat. Sci. 48: 1220–1225.

Sensitivity to low pH in freshwater fish appears to be correlated with density and replacement rates of gill chloride cells, but these cells are difficult to examine. Mayfly nymphs possess cells of similar appearance and probable function and are easily observed in dense accumulations on gill surfaces. We examined chloride cell density on the gills of nymphs of seven species of mayflies (two Heptageniidae, two Leptophlebidae, one Siphlonuridae, and two Baetidae). Although tolerance of low pH exposure varied considerably among the seven species, it was not correlated with gill area or chloride cell density. Gills of nymphs from soft water also had no greater chloride cell density than gills of nymphs of the same species from hard water. Finally, nymphs of the two species able to withstand 2 wk exposure to low pH did not show an increase in chloride cell density. It remains possible that the prolonged ionoregulatory stress of low pH results in cell damage rather than cell proliferation in sensitive species.

La sensibilité à un faible pH chez les poissons dulcicoles semble corrélée à la densité et au taux de remplacement des cellules à chlorures des branchies, mais ces cellules sont difficiles à examiner. Les nymphes d'éphémères possèdent des cellules d'apparence semblable et dont la fonction est probablement la même et sont faciles à observer car elles forment des accumulations denses à la surface des branchies. Nous avons examiné la densité des cellules à chlorures sur les branchies de nymphes de sept espèces d'éphémères (deux Heptageniidae, deux Leptophlebidae, un Siphlonuridae et deux Baetidae). La tolérance d'exposition à un pH faible variait considérablement parmi les sept espèces, mais elle n'était pas corrélée avec la superficie des branchies ni avec la densité des cellules à chlorures. La densité de ces cellules n'était pas plus grande dans les branchies des nymphes vivant en eau douce que dans celles des mêmes espèces trouvées en eau dure. Enfin, les nymphes des deux espèces capables de supporter une exposition de 2 sem à un faible pH n'ont pas montré d'augmentation de la densité des cellules à chlorures. Il est toutefois possible que le stress prolongé occasionné par un faible pH sur l'ionorégulation cause des dommages aux cellules plutôt qu'une prolifération de ces cellules chez les espèces sensibles.

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Among fish and arthropods, death resulting from low pH exposure is most likely due to ionoregulatory failure or blood/hemolymph acidosis (Wood and Rogano 1986). Chloride cells of fish gills may provide protection for exposed fish from the osmoregulatory stress of dilute soft water and/or low pH. The cells are large, rich in mitochondria, endoplasmic reticulum, and Na,K-ATPase, have highly specialized apical membranes, and appear to be involved in regulating Na^+, K^+, and Ca^{2+} balance (Karnaky et al. 1976; Perry and Walsh 1989). Chloride cells occur in greater density in populations of fish from naturally acidified lakes (Chevalier et al. 1985), in fish experimentally exposed to low pH (Tietge et al. 1988), and in fish transferred from hard to soft water or to low-pH water (Leino et al. 1987). Yellow perch (*Perca flavescens*), one of the most acid-tolerant species, is unusual in having sequestered clusters of chloride cells which remain undamaged at low pH (Leino et al. 1987). Although there is no direct evidence of the function of chloride cells in freshwater fish, sensitivity to low pH appears to be correlated with the position, density, and replacement rate of these cells.

Aquatic insects also have tissues specialized for ionoregulation (Komnick 1977). Chloride or ionoregulatory cells are characteristic of mayfly and stonefly nymphs and they occur on the gills and scattered over various parts of the cephalothorax and femurs (Filshie and Campbell 1984; Kapoor 1985). Chloride cells on the gill surfaces are unobstructed by other cells, and all existing chloride cells can be readily counted. Because of the relative abundance and diversity of mayfly species in lakes and streams in acid-sensitive areas, because of the variation in tolerance which recent field and laboratory work has established (Berrill et al. 1987; Peterson et al. 1985; Mackay and Kersey 1985; Hall and Ide 1987; Rowe et al. 1989), and because the nymphs possess easily examined and countable chloride cells which may be implicated in acid tolerance, mayflies are particularly attractive for further examination. We tested further the hypothesis that chloride cells permit organisms to survive in acid-stressed water. Using mayfly nymphs as our experimental organisms, we predicted that (1) nymphs of a species living in soft water should have a greater density of chloride cells than nymphs of the same species living in hard water, (2) the chloride cells of nymphs stressed by prolonged laboratory exposure to low pH should increase in density if the nymphs molted during the exposure, and (3) gills of nymphs of acid-tolerant species should have a greater density of chloride cells than gills of nymphs of sensitive species.

Methods

We collected late instar nymphs of seven species of mayflies (two Heptageniidae, two Leptophlebiidae, one Siphloneuridae,

TABLE 1. Mayfly species exposed to pH 4.0. All nymphs were collected from Haliburton Co. except where noted.

Species	Site collected	Experimental exposure	Sensitive/tolerant (references[a])
Leptophlebiidae			
Leptophlebia cupida			
Late instars	Indian R., Peterborough Co.	Nov., 10°C, static	Tolerant (1, 2, 3)
	Dickie L. inflow	Apr., 10°C, static	
	Silent L.	Apr. 10°C, static	
	French R., Sudbury Co.	Apr., 10°C, static	
Early instars	Dickie L.	Sept., 15°C, static	
Habrophlebia vibrans	Hollow R.	June, 15°C, recirculator	Sensitive (1)
	Jackson's Cr., Peterborough Co.		
Heptageniidae			
Stenonema vicarium	Hollow R.	June, 15°C, recirculator	Sensitive (1)
	Jackson's Cr., Peterborough Co.		
Epeorus pleuralis	Hollow R.	June, 15°C, recirculator	Genus sensitive (4)
Siphlonuridae			
Isonychia rufus			
Early to late instars	Madawaska R.	Aug., 15°C, recirculator	*I. bicolor* sensitive (1)
	Indian R., Peterborough Co.		
Baetidae			
Baetis flavistriga	Hollow R.	June, 15°C, recirculator	Sensitive (1, 4)
Pseudochleon sp.	Hollow R.	June, 15°C, recirculator	

[a]References: (1) Hall and Ide 1987; (2) Berrill et al. 1987; (3) Rowe et al. 1989; (4) Peterson et al. 1985.

TABLE 2. Number of *L. cupida* and *S. vicarium* surviving 2 wk of exposure to pH 4.0 and pH 6.5.

			Number surviving 2 wk					
			pH 4.0 trials			pH 6.5 trials		
Species	Stage	n·trial^{-1}	1	2	3	1	2	3
L. cupida (10°C)	Wing pad	35	35	35	35	35	35	35
	Middle instar	16	16	16	16	16	16	16
	Early instar	25	25	24	23	25	25	24
S. vicarium (15°C)	Wing pad	20	20	19	20	18	20	20

TABLE 3. Emergence of wing padded nymphs of *L. cupida* and *S. vicarium* warmed to 20°C following 2 wk of exposure to pH 4.0 and 6.5.

		Number emerging within 1 wk			
		pH 4.0 trials		pH 6.5 trials	
Species	n·trial^{-1}	1	2	1	2
L. cupida	10	10	9	8	9
	20	14	12	15	12
S. vicarium	16	15	14	16	14

and two Baetidae) from softwater streams on the Canadian Shield in south-central Ontario and nymphs of four of these species from hardwater streams south of the Shield. We also collected early and middle instars of two of the large species (Table 1).

To assess comparative sensitivity of the seven species to low pH, we exposed nymphs from softwater streams to pH 4 under laboratory conditions. Nymphs were placed in experimental containers within 6 h of collection. Soft water (pH 6.5) for the exposures was collected from Paint Lake in Haliburton Co. and filtered (1 μm). The chemical composition (millimolar) of this water was $Ca^{2+} = 0.10$, $Na^{+} = 0.10$, $Cl^{-} = 0.9$, $K^{+} = 0.02$, $Mg^{2+} = 0.06$, $SO_4^{2-} = 0.08$, and $Al^{3-} = 0.001$. Nymphs of swift-water species were placed in 1.5-L screened containers in recirculators based on the design described by Vogel and LaBarbera (1978). Nymphs of slower water species (*Leptophlebia cupida* and *Stenonema vicarium*) were placed in brain jars containing 3 L of water. Recirculators and static water jars were kept in controlled-temperature environmental chambers. In each experiment, pH was increased gradually over 6–8 h in half the containers to 4.0 and adjusted daily, if necessary, with 0.1 N H_2SO_4 and 0.1 N NaOH. Daily variation rarely was greater than 0.1 pH unit. Water was aerated throughout exposure periods. Exposures lasted for up to 2 wk but were terminated earlier if all nymphs exposed to pH 4 died. As many as 20 nymphs were placed in each screened container or brain jar, and experiments were run in duplicate (in recirculators) or triplicate (in static water jars). Dead nymphs and molted exuviae, if any, were removed daily.

We counted the chloride cells on the gills of late instar nymphs of all seven species, as well as middle and early instars

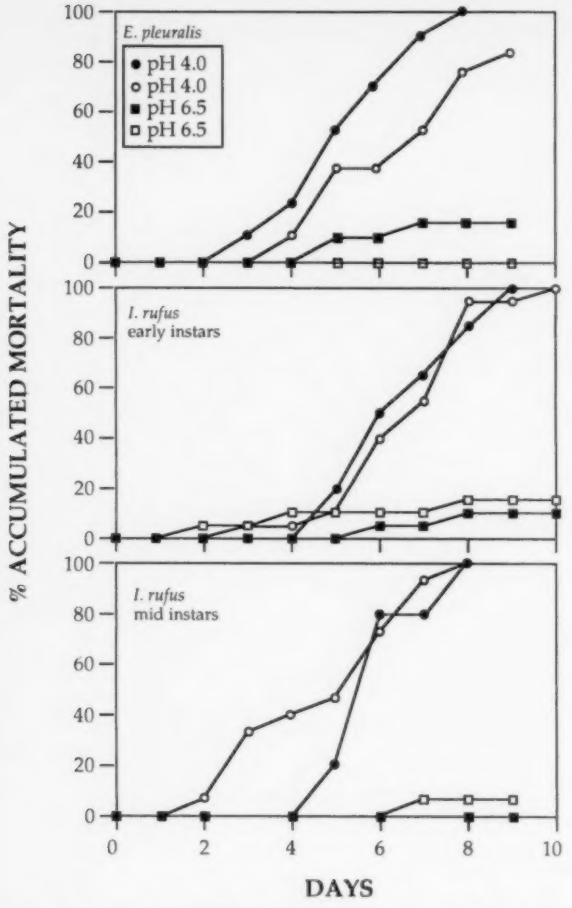

FIG. 1. Percent accumulated mortality of late instar nymphs of *E. pleuralis* (15 per replicate), early instars of *I. rufus* (20 per replicate), and middle instars of *I. rufus* (16 per replicate).

of *L. cupida* and *Isonychia rufus*. Gills were from freshly caught nymphs, with the exception of those surviving exposure to pH 4.0 (and their controls at pH 6.5) for a 2-wk period. To stain chloride cells, we immersed at least 10 live nymphs in soft water to which we added an equal amount of a 4% solution of $AgNO_3$ and then exposed the nymphs to a bright light for 20–30 min before preserving them in 70% alcohol. We counted the chloride cells on one surface of the largest gill of each nymph and measured gill area using an optical grid and 10× microscope objective. Gills of several species were prepared for scanning electron microscopy (SEM) by sputter coating (100 Å) dehydrated nymphs and were photographed at up to 2000× using a Cambridge 600 SEM.

Results

Comparative Sensitivity to Low pH

The seven species exposed for 2 wk to pH 4.0 varied considerably in sensitivity. Nymphs of *L. cupida* (Leptophlebiidae) and *S. vicarium* (Heptageniidae) survived exposure with little or no mortality (Table 2). When late instar nymphs of both species were raised to 20°C for an additional 1-wk period, over 80% emerged successfully, again indicating tolerance of low pH exposure (Table 3). *Epeorus pleuralis* (Heptageniidae) and *I. rufus* (Siphlonuridae) were of intermediate sensitivity. After 4 d of exposure to pH 4, mortality of *E. pleuralis* nymphs increased rapidly. Six of 30 nymphs actually emerged successfully during the first week of exposure, but none of the remainder survived past the ninth day. Wing-padded nymphs of *I. rufus* did not live for more than 2–3 d even at pH 6.5 and were therefore discarded from the experiment; they probably needed a swifter water current than we could generate in the recirculators. However, at least 90% of middle and early instars survived at pH 6.5, while the mortality of those exposed to pH 4.0 increased gradually until the last died after 11 d (Fig. 1). Nymphs of *Baetis flavistriga*, *Pseudochleon* sp. (Baetidae), and *Habrophlebia vibrans* (Leptophlebiidae) all died within 1–5 d of exposure to pH 4.0 (Fig. 2).

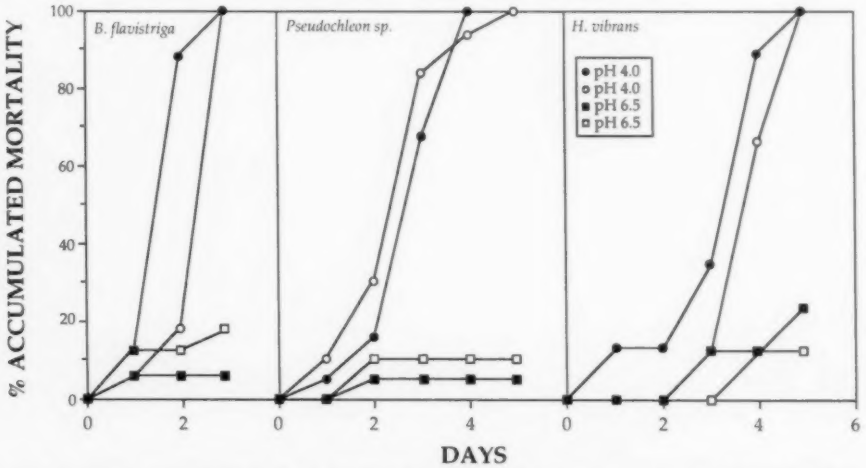

FIG. 2. Percent accumulated mortality of late instar nymphs of the three mayfly species most sensitive to pH 4 exposure. Sample sizes per replicate were 16 for *B. flavistriga*, 20 for *Pseudochleon* sp., and 10 for *H. vibrans*.

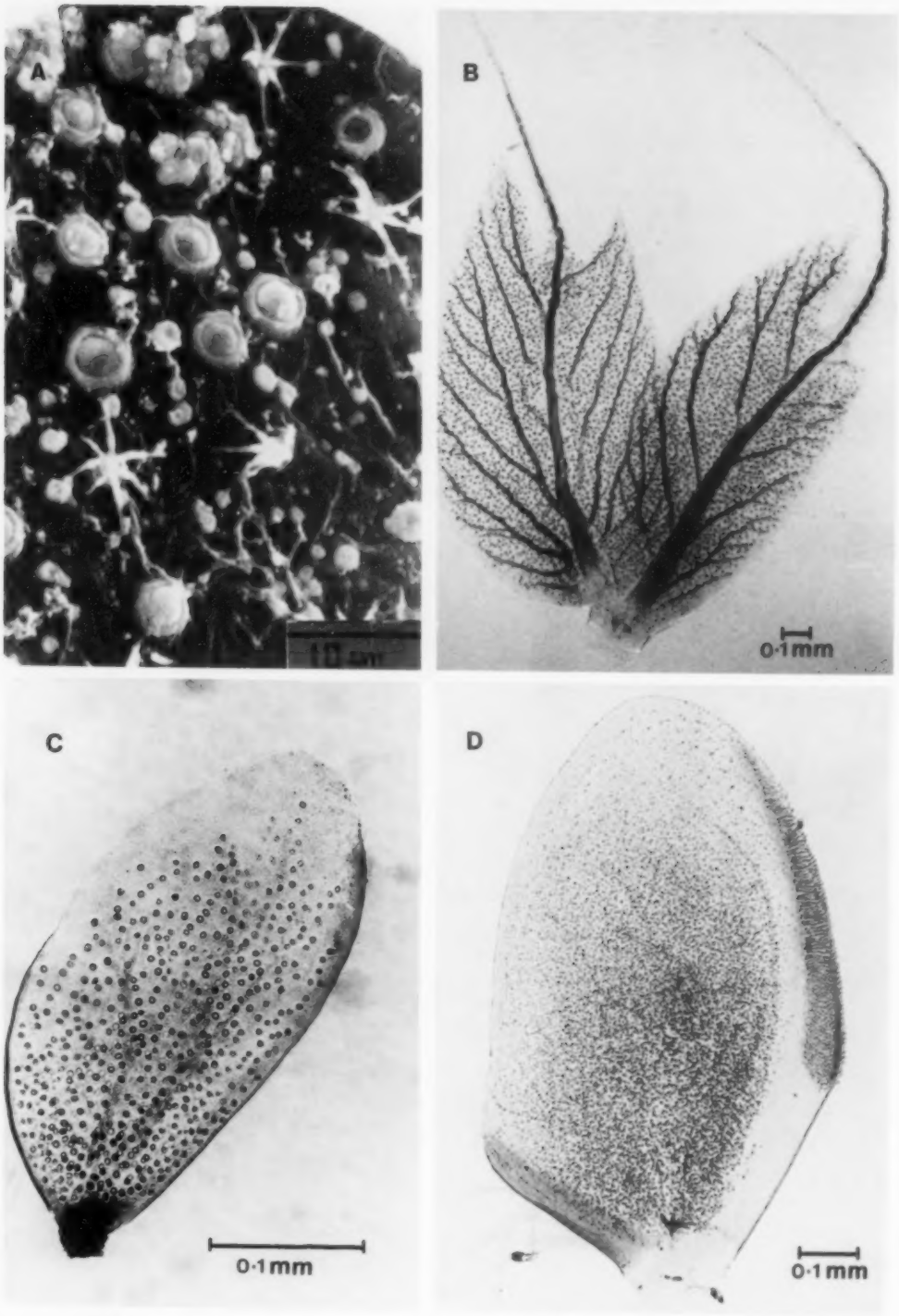

FIG. 3. Chloride cells on gills of mayfly nymphs. (A) Scanning electron micrograph of chloride cells of *E. pleuralis* showing apical surfaces of both coniform (donut shaped) and filiform (star shaped) chloride cells. (B) Fourth abdominal gill of *L. cupida*: chloride cells are black dots, stained with $AgNO_3$. (C) Fourth abdominal gill of *Pseudochleon* sp. (D) Fourth abdominal gill of *E. pleuralis*.

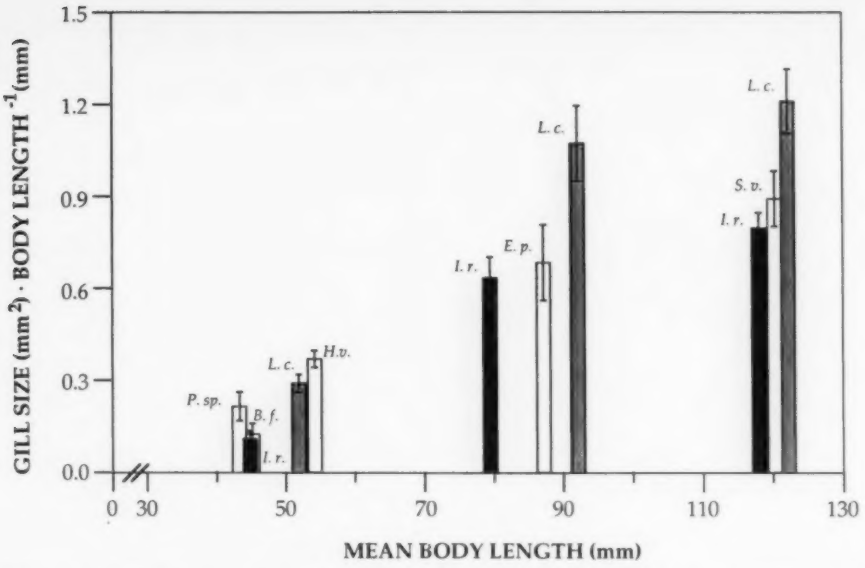

FIG. 4. Relationship between gill size to body length and body length of nymphs of seven species of mayflies. *B.f.* = *B. flavistriga*, *E.p.* = *E. pleuralis*, *H.v.* = *H. vibrans*, *I.r.* = *I. rufus*, *L.c.* = *L. cupida*, *P.sp.* = *Pseudochleon* sp., and *S.v.* = *S. vicarum*. Nymphs are all late instars except for early and middle instars included for *I. rufus* and *L. cupida*. Error bars indicate standard deviations. Sample size in all cases = 10.

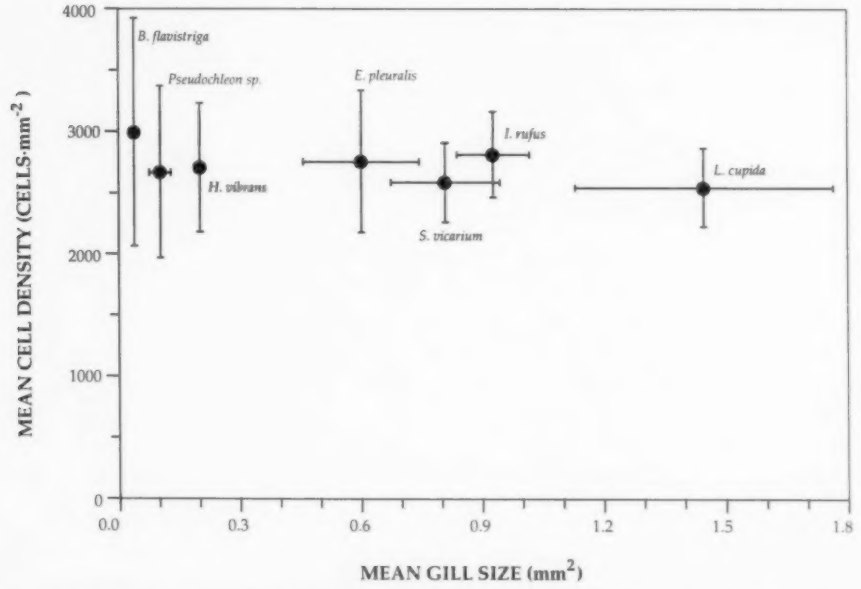

FIG. 5. Relationship between chloride cell density and gill size of nymphs of seven species of mayflies. ANOVA indicated no significant differences in mean cell densities (df = 6, $F = 1.07$, $p > 0.05$). Sample sizes in all cases = 10.

Chloride Cells on Gills

While gills varied in size and shape, chloride cells were distributed on both surfaces of each abdominal gill of all stages of all nymphs (Fig. 3). Most chloride cells appear as donuts or rings on the gill surface. Examination under SEM indicates that bulging apical membranes characterize these cells (coniform chloride cells, Komnick 1977); other less common filiform chloride cells have projections extending from their apical membranes (Fig. 3).

Larger nymphs have relatively larger gills; the gills of middle and late instar *L. cupida* are particularly large (Fig. 4). The four tolerant or moderately tolerant species (*L. cupida*, *S. vicar-*

ium, *I. rufus*, and *E. pleuralis*) grow considerably larger than the sensitive species. Since the small instars of the large species are as tolerant of low pH exposure as the larger instars, there is therefore no correlation between relative gill size and acid tolerance. As a result, if chloride cells protect acid-tolerant species from exposure to low pH, the gills of tolerant species should have greater densities of chloride cells.

Relationship of Chloride Cell Density to Gill Size

There was no significant difference in the density of chloride cells on gills of late instar nymphs of all seven species collected from softwater streams, although considerable natural variation exists (Fig. 5).

Relationship of Chloride Cell Density to Water Hardness and pH

To test whether nymphs living in softwater have a greater density of chloride cells than nymphs from hard water, we examined four species, *L. cupida*, *S. vicarium*, *H. vibrans*, and *I. rufus*. In each species, there was no significant difference in chloride cell density on gills of nymphs from the two water types (*t*-tests: *L. cupida*, df = 18, $t = 0.93$, $p > 0.05$; *S. vicarium*, df = 18, $t = 0.42$, $p > 0.05$; *H. vibrans*, df = 18, $t = 1.34$, $p > 0.05$; *I. rufus*, df = 18, $t = 1.01$, $p > 0.05$).

To test whether chloride cells proliferate at low pH, we examined gills of *L. cupida* and *S. vicarium* nymphs exposed for 2 wk to pH 4.0. Although a high rate of molting occurred in both exposures (>90%), allowing opportunity for cell proliferation, chloride cell density on gills of nymphs exposed to low pH was not significantly different than cell density on gills of nymphs exposed to pH 6.5 (*t*-tests: *S. vicarium*, df = 18, $t = 1.21$, $p > 0.05$; *L. cupida*, df = 18, $t = 0.54$, $p > 0.05$).

Discussion

General agreement between our laboratory results and field studies (Hall and Ide 1987; Peterson et al. 1985; Fiance 1979) in classifying sensitivity to pH gives us confidence to accept the seven mayfly species as representative of the natural variation. We also conclude that none of our three initial predictions concerning changes in chloride cell distribution is upheld. First, late instar nymphs of species living in soft water have no more gill chloride cells than nymphs living in hard water. Second, nymphs exposed to low-pH soft water do not increase the number of their chloride cells despite molting during the exposure period. Finally, nymphs of acid-tolerant and moderately tolerant species do not have more chloride cells than similarly sized nymphs of acid-sensitive species. No clear correlation appears to exist between acid tolerance and the density and/or number of chloride cells on the gills of mayfly nymphs.

It remains likely that the chloride cells of mayfly gills are involved in ionoregulation. Chloride cell morphology, distribution, associated enzymes, and, in one species (Wichard et al. 1973), proliferation when exposed to very soft water all support this conclusion. However, our results indicate no similar proliferation of chloride cells as an adaptative response to the ionic stress of soft water or of low-pH conditions. It remains possible that the prolonged ionoregulatory stress of low pH results in cell damage rather than cell proliferation. As well, other branchial mechanisms may be involved in ion and acid–base regulation, as recently proposed for rainbow trout gills (McDonald and Prior 1988). Alternatively, chloride cell density on mayfly gills may be in excess of what is necessary to respond to ionoregulatory challenge, and proliferation of cells is unnecessary. Mayfly chloride cells remain easily accessible for analysis, however, and should provide an alternative to the chloride cells of fish for further experimentation.

Acknowledgements

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Size-Dependent Ammonium and Phosphate Uptake, and N:P Supply Ratios in an Oligotrophic Lake[1]

Curtis A. Suttle

Marine Science Institute, University of Texas at Austin, Port Aransas, TX 78373-1267, USA

William P. Cochlan

Scripps Institute of Oceanography, Marine Biology Division, 0202, University of California at San Diego, La Jolla, CA 92093-0202, USA

and John G. Stockner

Department of Fisheries and Oceans, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6, Canada

Suttle, C. A., W. P. Cochlan, and J. G. Stockner. 1991. Size-dependent ammonium and phosphate uptake, and N:P supply ratios in an oligotrophic lake. Can. J. Fish. Aquat. Sci. 48: 1226–1234.

One arm of oligotrophic Sproat Lake was fertilized with weekly additions of inorganic phosphorus and nitrogen (N:P = 50:1, by atoms). There was a lag of greater than 1 mo before changes in N:P supply ratio were reflected by "relative uptake rates" (RURs) of saturating ammonium and phosphate additions. This supports previous findings that RUR is a sensitive indicator of changes in N:P supply ratio, but suggests that changes in RUR are mediated by changes in composition rather than physiological state of the plankton community. The <3 μm fraction showed a greater demand for phosphate than ammonium compared with larger cells (>3 μm), as indicated by maximum uptake rates for the two nutrients. However, maximum phosphate uptake rates for both size fractions were greater as the result of fertilization. Nonetheless, RURs for both size fractions were consistent with *Synechococcus* being N limited and diatoms being P limited when the lake was not fertilized. Despite the large increase in nutrient loading, dissolved phosphate turnover times decreased from 10–12 to 5–7 min, indicating increased phosphate demand relative to supply. This study emphasizes that ammonimum and phosphate kinetics data yield valuable information on the nutritional conditions experienced by phytoplankton in lakes.

Un bras du lac Sproat, lac oligotrophe, a été fertilisé par des ajouts hebdomadaires de phosphore et d'azote inorganiques (N :P = 50:1, en nombre d'atomes). Il a fallu attendre plus de 1 mo avant que les modifications du rapport N:P se manifestent dans les taux relatifs d'absorption (RUR) des ajouts d'ammonium et de phosphate à saturation. Cette observations vient confirmer des résultats antérieurs qui indiquaient que le RUR est un indicateur sensible des changements dans le rapport N:P, mais laisse penser que les changements du RUR passent par des modifications de la composition plutôt que de l'état physiologique de la communauté planctonique. La fraction < 3 μm montrait une demande plus forte de phosphate que d'ammonium par rapport aux cellules plus grandes (> 3 μm), comme l'indiquent les taux maximum d'absorption des deux nutriants. Toutefois, les taux maximum d'absorption du phosphate dans les deux fractions de taille étaient plus élevés à la suite de la fertilisation. Les RUR pour les deux fractions de taille étaient conséquents : *Synechococcus* était N limité et les diatomées étaient P limitées quand le lac n'était pas fertilisé. Malgré la forte augmentation de la charge en nutriants, le temps de renouvellement du phosphate dissous a baissé, passant de 10–12 à 5–7 min, ce qui indique une augmentation de la demande de phosphate comparativement à l'apport. Notre étude souligne que les données sur la cinétique de l'ammonium et du phosphate apportent de l'information précieuse sur la situation nutritionnelle du phytoplancton dans les lacs.

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Evidence from laboratory (Tilman et al. 1986; Suttle and Harrison 1988a) and field studies (Schindler 1977; Stockner and Shortreed 1988) demonstrates that relative supply rates of nitrogen and phosphorus can affect the structure of phytoplankton communities, thereby potentially affecting pathways of nutrient and energy transfer. For undisturbed natural freshwater systems, however, the paradigm has been that phytoplankton biomass in lakes is controlled solely by P availability (Schindler 1977). This view is changing as evidence mounts that both N and P are important in limiting phytoplankton biomass in lakes (reviewed by Elser et al. 1990). In fact, N and P can limit different components of phytoplankton communities simultaneously (Suttle and Harrison 1988a; Wehr 1989), which should make community structure particularly sensitive to changes in the N:P supply ratio.

In order to predict how changes in N or P loading might affect phytoplankton community composition, it would be beneficial to be able to monitor in situ N:P supply ratios prior to and in response to changes in N and P loading regimes. Unfortunately, this is not a trivial problem. However, as nutrient uptake kinetics are sensitive to the composition and physiological status of phytoplankton communities, in situ N:P supply ratios can be

[1]Contribution No. 812 of The Marine Science Institute, University of Texas at Austin.

estimated from "relative uptake rates" (RURs) of ammonium and phosphate (Suttle and Harrison 1988a).

As well, it has been postulated that in lakes where both N and P potentially limit autotrophic growth the *Synechococcus*-dominated size fraction (<3 μm) may be N limited because of its large requirement for N relative to P, while larger cells (mainly diatoms) may be P limited (Suttle et al. 1988). One way to address this hypothesis is to examine the relative uptake rates (RURs) of ammonium and phosphate in the <3 and >3 μm size fractions and compare these with RURs collected for individual species of phytoplankton grown under different degrees of N and P limitation in the laboratory. From these data the relative importance of N versus P limitation in these two size classes can be inferred.

The presence of fertilized and unfertilized regions in oligotrophic Sproat Lake, which were isolated from each other by shallow sills and the direction of water flow but otherwise were similar, provided an opportunity to examine these hypotheses more closely. Our study examined the magnitude and time scale of the response of the RUR index to changes in N:P supply ratio caused by fertilization of the lake, and ammonium and phosphate kinetics to determine if the data were consistent with N limitation of *Synechococcus* (<3 μm) and P limitation of the larger diatoms (*Cyclotella* and *Rhizosolenia*). As well, we examined the effects of fertilization on chlorophyll-specific ammonium and phosphate uptake rates of the <3 and >3 μm size fractions and on phosphate turnover times.

Methods

Sproat Lake (surface area 41 km^2), located on Vancouver Island in southwestern British Columbia, Canada, is oligotrophic and warm-monomictic, mixing completely from about November to March. The lake, with an average depth of about 59 m and an estimated water residence time of 8 yr, is described in further detail elsewhere (Shortreed and Stockner 1990). On a weekly basis from 20 May to 7 July 1986, a converted DC6-B water bomber was used to enrich the most western arm (Taylor Arm) of the lake with an aqueous solution of ammonium phosphate and ammonium nitrate (N:P = 50:1, by atoms) for the purpose of increasing sockeye salmon (*Oncorhynchus nerka*) production (Stockner 1981). These additions increased the weekly phosphate load to this arm by about 180 μmol $P \cdot m^{-2}$, approximately doubling the estimated annual P input to the lake (J. G. Stockner, unpubl. data).

Water samples (20 L) for all experiments were collected from 1 m at two stations (Fig. 1) and flown to Vancouver in two carboys packed in insulated boxes. The fertilized (west) and unfertilized (south) arms of the lake were sampled monthly from May through September 1986 (Table 1). Samples were collected the day following fertilization except in May when the samples were collected 3 d later. Transporting of samples by air prevented shipment of more than one container of water from each station; hence, replicate determinations were for subsamples taken from each carboy, yielding estimates of the precision of our experimental protocols, but not of the variation within the fertilized and unfertilized arms.

Uptake Experiments

Ammonium and phosphate uptake experiments were carried out on each sampling date. The water was filtered through 120-μm Nitex screening to remove large zooplankton and duplicate 2.5- and 0.5-L samples were dispensed into 3- and 1-L Pyrex flat-bottomed boiling flasks. Experiments were initiated within 3.5–4.5 h of collecting the water by adding isotopically labelled phosphate or ammonium at concentrations saturating to uptake. The appropriate phosphate and ammonium concentrations required to saturate uptake rates were previously determined (Suttle and Harrison 1988b) during studies on a similar nearby lake (Kennedy Lake). Incubations were at lake temperature (Table 1) at an irradiance of approximately 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Westinghouse "Super-Hi" Output Daylight fluorescent bulbs) for 2 h. Experiments were terminated by filtration at a pressure differential of <100 mm Hg (1 mm Hg = 133.322 Pa) and uptake rates were calculated from the incorporation of isotope into particulate material.

For the PO_4^{3-} uptake experiments, K_2HPO_4 was added to the 0.5-L samples to increase the concentration by 2.0 μM and immediately labelled with 0.2-μm-filtered carrier-free $^{32}PO_4^{3-}$ to approximately 30 000 $dpm \cdot mL^{-1}$. For determination of phosphate turnover times, carrier-free $^{32}PO_4^{3-}$ was added to duplicate 0.5-L samples and 5-mL aliquots were filtered at 2-min intervals over a 30-min period. Turnover times were calculated from semi-log plots of the percentage of ^{32}P remaining in the filtrate versus the time of the incubation. The regression line describing the initial slope multiplied by 2.303 is an estimate of the uptake constant (in units of $time^{-1}$), the reciprocal of which is the turnover time of the ambient phosphate pool (Lean and Rigler 1974; Peters 1978). Phosphate uptake experiments were terminated by filtration through 0.2- or 3.0-μm Nuclepore polycarbonate filters; uptake in the 0.2–3.0 μm fraction was determined by difference. Nuclepore filters were always placed over glass-fiber filter underlays and the vacuum maintained while the polycarbonate filters were removed. This alleviated the need for rinsing and resulted in little retention of isotope-labelled water by the filter and consistently low blanks. The amount of ^{32}P in the filtered samples was determined using a liquid scintillation counter after dissolving the filters in 200 μL of a 10:1 mixture of methylene chloride and ethanolamine.

Samples (2.5 L) for determining NH_4^+ uptake rates were incubated in the presence of 10 μM $^{15}NH_4Cl$ (99 atom%) except for those collected in September when 15 μM $^{15}NH_4Cl$ was added. At the end of the NH_4^+ uptake experiments, samples were either filtered directly onto combusted Whatman GF/F filters or, in order to determine uptake by the <3.0 μm fraction, prefiltered through 3.0-μm Nuclepore filters before being filtered onto GF/F filters. The ^{15}N enrichment of the samples was assayed by emission spectrometry (Jasco model N-150) after conversion of the particulate N to N_2 gas by the micro-Dumas dry combustion technique as outlined by La Roche (1983). Ammonium uptake rates were calculated using the equation of Dugdale and Wilkerson (1986) which corrects for changes in particulate N during the incubation.

Nutrient Chemistry

Lake water for dissolved nutrient analyses was gently filtered through combusted GF/F filters (450°C for 6 h), which were rinsed with at least 100 mL of lake water before samples were collected. Automated techniques as outlined in Stainton et al. (1977) were used for determination of ammonium by the phenol-hypochlorite method, nitrate by cadmium reduction to ammonium, and total dissolved nitrogen (TDN) by UV oxidation and conversion to ammonium. For chlorophyll, partic-

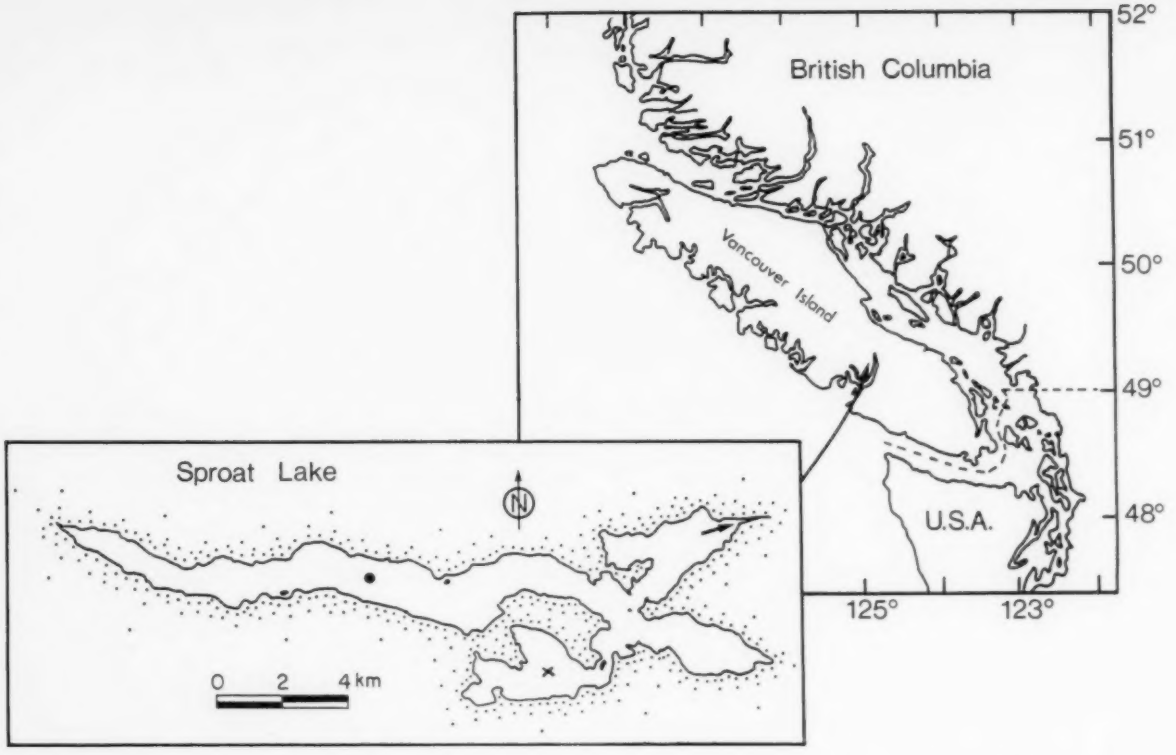

FIG. 1. Sampling stations in fertilized Taylor Arm (●) and unfertilized Two Rivers Arm (×), Sproat Lake, Vancouver Island, southwestern British Columbia.

TABLE 1. Temperature (°C), Chl ($\mu g \cdot L^{-1}$), and nutrient (μM) data from the 1-m depth of the fertilized (Fert) and unfertilized (Unfert) arms of Sproat Lake (PC, particulate carbon; PN, particulate nitrogen; PP, particulate phosphorus; TDN, total dissolved nitrogen). Concentrations are means of duplicate or triplicate determinations, except where noted. Significant differences between samples from fertilized and unfertilized arms were demonstrated using *t*-tests. The lake was fertilized from 20 May to 7 July. $*p < 0.05$; $**p < 0.01$.

	16 May		10 June		8 July		5 Aug.		17 Sept.	
	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert	Fert	Unfert
Temperature	10.5	11.5	17.0	17.0	18.0	19.5	24.5	23.0	19.0	20.0
Chl(total)	0.50	0.48	0.31	0.38	1.03	0.28**	0.21	0.24	1.25	0.56
Chl (<3 μm)	0.15	0.13	0.12	0.18	0.40	0.12*	<0.12	<0.12	1.05	0.37*
PC(total)	9.6	13.2*	7.1	8.7	23.4	11.9*	16.8	15.3	22.6	17.5
PC(<3 μm)	10.3	5.0*	2.0	4.3	10.8	3.0*	8.0	9.4	7.8	8.8
PN(total)	1.09	1.32	1.14	1.35	3.00	1.45*	1.55	1.47	1.98	1.51
PN(<3 μm)	0.94	0.96	0.52	0.88**	1.82	0.81*	0.96	0.99	0.82	0.70
PP(total)	0.019[a]	0.016[a]	0.028	0.066*	0.071	0.095	0.026	0.030	0.032	0.019
PP(<3 μm)			0.026	0.066	0.026	<0.030	0.032	0.030		
NH_4^+	<0.29	<0.29	<0.29	<0.62[c]	0.64[b]	0.64[b]	0.46	<0.36[c]	<0.29	<0.29
NO_3^-	0.57	0.20**	0.50	1.48**	0.64[b]	<0.14[b]	0.11	<0.07	<0.07	0.19
Total P	0.05[a]	0.03[a]	0.06[a]	<0.03[a]	0.10[a]	<0.03[a]	<0.03	<0.03	<0.03	0.05
TDN	16.0[a]	9.0[a]	12.4[b]	11.2[b]	9.9[b]	6.3[b]	7.6	6.9	10.1	9.3

[a]Data from approximate dates shown here.
[b]Single determination.
[c]One replicate was less than the detection limit (0.29 μM).

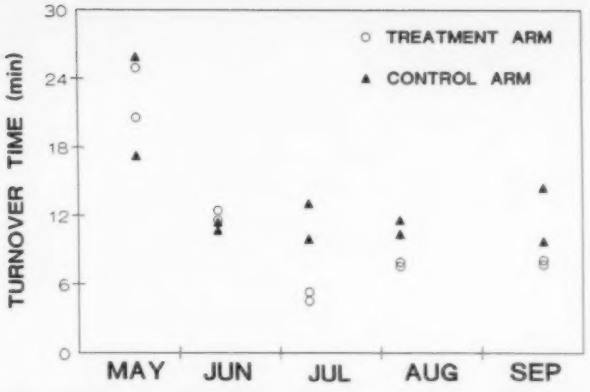

FIG. 2. Duplicate determinations of turnover times of the dissolved orthophosphate pool in the fertilized (treatment) and unfertilized (control) regions of Sproat Lake on each of the sampling dates. Fertilization commenced on 20 May, 2 d after the first sampling date, and ceased on 7 July.

ulate carbon (PC), particulate nitrogen (PN), and particulate phosphorus (PP) duplicate 1-L samples were collected, filtered, and size-fractionated as outlined above for the ^{15}N samples. Chlorophyll *a* was analyzed fluorometrically (Strickland and Parsons 1972), PC and PN were analyzed by the dry combustion method of Sharp (1974) using a Perkin-Elmer elemental analyzer, and PP was determined as described in Stainton et al. (1977).

Phytoplankton Abundance

The phytoplankton data are presented as averages of counts from the epilimnion of the lake (1, 3, 5, and 10 m). Phytoplankton >3 μm were identified and enumerated in whole lake water which was settled and then counted using a Wild inverted microscope equipped with phase-contrast optics. For enumeration of picoplankton, whole lake water was filtered onto an Irgalan black stained 0.2-μm Nuclepore filter at a vacuum of <150 mm Hg, and autofluorescent cells <3 μm were counted using a Zeiss epifluorescence microscope (Hobbie et al. 1977).

Results

Concentrations of particulate and dissolved nutrients were not higher in the treated arm after 3 wk of fertilization (10 June, Table 1); however, by the July sampling date, concentrations of chlorophyll *a*, PC, and PN were significantly higher ($p < 0.05$). Yet, within 4 wk after nutrient additions had ceased (7 July), there were no significant differences in concentrations between the treated and untreated arms, except for higher chlorophyll *a* concentrations in the <3 μm size class during September. This indicated a larger fall bloom of small phytoplankton in the arm which had been fertilized.

Dissolved phosphate turnover times (Fig. 2) were also similar between the two arms of the lake on the first two sampling dates. Prior to fertilization in May they were about 22 min, but decreased to about 12 min in June after 3 wk of fertilization. Subsequently the data diverged; phosphate turnover times remained similar in the unfertilized arm, but were significantly less ($p < 0.01$, ANOVA) in the fertilized portion of the lake. Unlike nutrient concentrations, differences in turnover times between arms persisted after fertilization ceased.

Maximum phosphate uptake rates (Table 2) were also similar between treated and untreated arms in May and June, but were about fourfold higher in the fertilized arm in July ($p < 0.01$) and 1.5 times higher in August ($p < 0.05$). By September the maximum phosphate uptake rates in both regions of the lake were again similar. Maximum ammonium uptake rates in the unfertilized and fertilized arms were not significantly different except in August and September when uptake in the fertilized arm was greater than that in the untreated arm. Samples for the May ammonium uptake data from the fertilized arm were lost.

RURs were similar for fertilized and unfertilized arms except on 8 July when the value for the treated arm was about fourfold that of the unfertilized arm (Table 2). Estimated N:P supply ratios calculated from RURs using the equation, N:P supply ratio = ln (RUR/0.0148)/0.1496 (Suttle and Harrison 1988a) ranged from 24 to 35 and 24 to 44 in the unfertilized and fertilized arms, respectively. RURs for the July date yielded estimated N:P supply ratios for the fertilized and unfertilized arms of 35 (80% CI = 29–39) and 44 (80% CI = 38–48), respectively.

Averaged over all dates the ratio of the maximum phosphate to the maximum ammonium uptake rates was greater for the <3 μm size class (Fig. 3; $p < 0.05$). In the unfertilized arm, RURs for the <3 μm size class ranged from 1.4 to 4.9 times greater than for the >3 μm size fraction, while in the fertilized arm the range was from 0.7 to 11.2 times. The August sampling date in the fertilized arm was the only time when the RUR was higher for the >3 than for the <3 μm size class.

The partitioning of the phosphate flux between the <3 and >3 μm fractions during saturating phosphate additions (Fig. 4) varied over the course of the sampling period, with more of the uptake by the small size fraction in May and June than in the remaining months. However, for any given sampling date the partitioning of phosphate flux between the two size classes remained similar in the treated and untreated arms, despite the differences in nutrient loading and phosphate turnover times between the two regions. The percentage of the ammonium taken up by the <3 μm size fraction was also similar between the two arms except for the June sampling date when uptake by the small size fraction in the fertilized arm was only about half of that in the unfertilized arm. As well, the partitioning of the ammonium and phosphate uptake between the two size fractions was not necessarily equal. For example, during June and September the <3 μm size fraction was responsible for a greater proportion of the phosphate uptake than the ammonium uptake.

The <3 μm size fraction had higher chlorophyll *a* specific saturated uptake rates for phosphate than the >3 μm size class in May and June (i.e. values >1.0), but less in August and September in both fertilized and unfertilized arms (Table 3). A similar pattern was observed for the uptake of ammonium. As well, fertilization resulted in higher relative chlorophyll *a* specific ammonium and phosphate uptake rates by the <3 μm size fraction on the June and July sampling dates ($p < 0.05$), but this effect did not persist beyond the period of fertilization.

Although the dominant phytoplankton in the epilimnion of the lake changed with time, for any given sampling date the same phytoplankton dominated both fertilized and unfertilized arms (Fig. 5). The diatom *Rhizosolenia* spp. was most abundant at the beginning of the sampling period and showed no pronounced response to fertilization. In comparison, the diatom *Cyclotella* spp. and the cyanobacterium *Synechococcus* spp. showed sizable increases in cell numbers in the treated over the

TABLE 2. Maximum phosphate and ammonium uptake rates ($nmol \cdot L^{-1} \cdot h^{-1}$) in the fertilized and unfertilized arms of Sproat Lake. The ratio of those uptake rates (RUR) was used to estimate the in situ supply ratio of N and P (see text). The asterisks indicate significant differences (*t*-test: $*p < 0.05$; $**p < 0.01$) in uptake rates between the fertilized and unfertilized arms; standard deviations (SD) and confidence intervals (CI) are given.

Date	Treatment	Saturated uptake rates		RUR (SD)	Estimated N:P supply ratio (80% CI)
		Phosphate (SD)	Ammonium (SD)		
16 May	Unfertilized	24.1 (0.3)	21.1 (0.6)	1.1 (0.04)	29 (23–33)
	Fertilized	23.5 (0.1)			
10 June	Unfertilized	11.7 (0.2)	21.5 (1.6)	0.55 (0.04)	24 (18–28)
	Fertilized	10.0 (0.5)	18.3 (0.4)	0.55 (0.03)	24 (18–28)
8 July	Unfertilized	94.3 (1.0)**	36.0 (3.9)	2.6 (0.29)	35 (29–39)
	Fertilized	408.7 (11.0)	37.4 (0.3)	10.9 (0.31)	44 (38–48)
5 Aug.	Unfertilized	67.0 (2.8)*	27.3 (0.0)*	2.5 (0.10)	34 (29–39)
	Fertilized	95.0 (0.2)	37.7 (0.7)	2.5 (0.05)	34 (29–39)
17 Sept.	Unfertilized	59.1 (6.4)	29.1 (5.0)*	2.0 (0.41)	33 (27–37)
	Fertilized	70.0 (2.1)	49.2 (0.3)	1.4 (0.04)	31 (25–35)

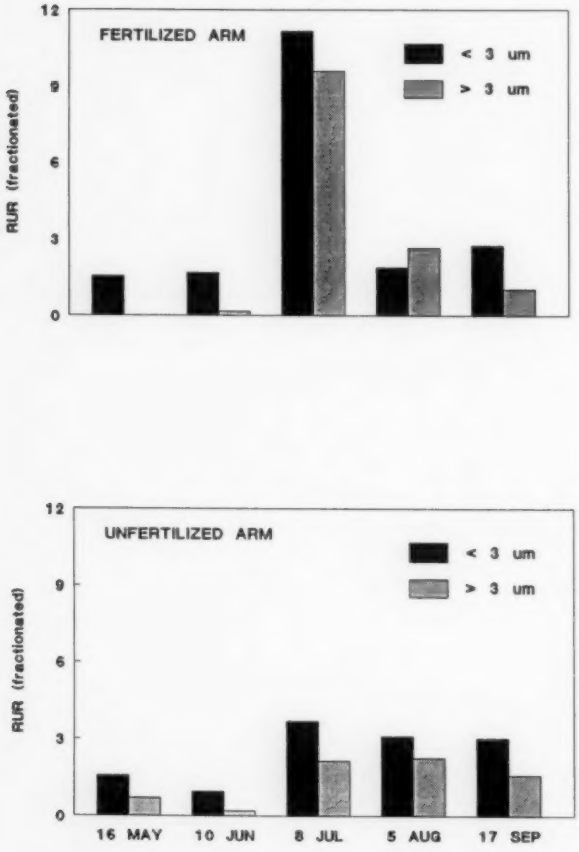

FIG. 3. Relative maximum phosphate to maximum ammonium uptake rates (RUR) for the <3 and >3 μm size classes from the unfertilized and fertilized arms of Sproat Lake.

untreated arm. *Cyclotella* numbers in the fertilized arm increased about threefold over those in the unfertilized and remained considerably elevated through to the end of July, 24 d after fertilization had ceased. *Synechococcus* was by far the most abundant phytoplankter in the fertilized and unfertilized regions of the lake and showed a much larger increase in absolute numbers as a result of the nutrient additions. Nonetheless, as a proportion of the original cell numbers the increases in *Cyclotella* and *Synechococcus* were similar. Unlike *Cyclotella*, however, numbers of *Synechococcus* decreased rapidly once the nutrient additions had ceased. The increases in abundance of *Cyclotella* and *Synechococcus* were coincident with similar increases in chlorophyll *a* in both the large and small size fractions (Table 1).

Discussion

A number of salient points emerge as the result of this study. First, RURs were sensitive to changes in the N:P supply ratio caused by fertilization of the lake, consistent with RUR being a measure of in situ N:P supply ratio. Second, changes in RUR were apparently mediated more by changes in species composition than physiological status of the plankton community. Third, RUR values suggest that cells in the <3 μm fraction (primarily *Synechococcus*) were N limited, while larger cells (primarily diatoms) were P limited. Fourth, despite an approximate doubling of the annual phosphate load to the fertilized arm, phosphate turnover times decreased, indicating that phosphate remained in short supply relative to demand.

N:P Supply Ratios

The N:P supply ratio can have a major influence on the composition of phytoplankton communities (Schindler 1977; Barcia et al. 1980; Stockner and Shortreed 1988), thereby affecting pathways of nutrient and energy flow. For example, nutrient loading to lakes must be controlled so that the overall N:P supply ratio is not decreased to the point where the competitive advantage is shifted to N-fixing cyanobacteria. This can be difficult because the overall nutrient load to a system may not be well defined, and the rates and efficiencies with which dissolved inorganic P and N recycle are variable. As RURs provide a means of estimating the overall N:P supply ratio in a system, without any knowledge of N and P loading, such an index should be particularly useful. If RURs are going to be used to assess the effect of changes in N and P loading on N:P supply ratios, it is essential to know the time scale on which changes in N:P supply ratio are going to be reflected by changes

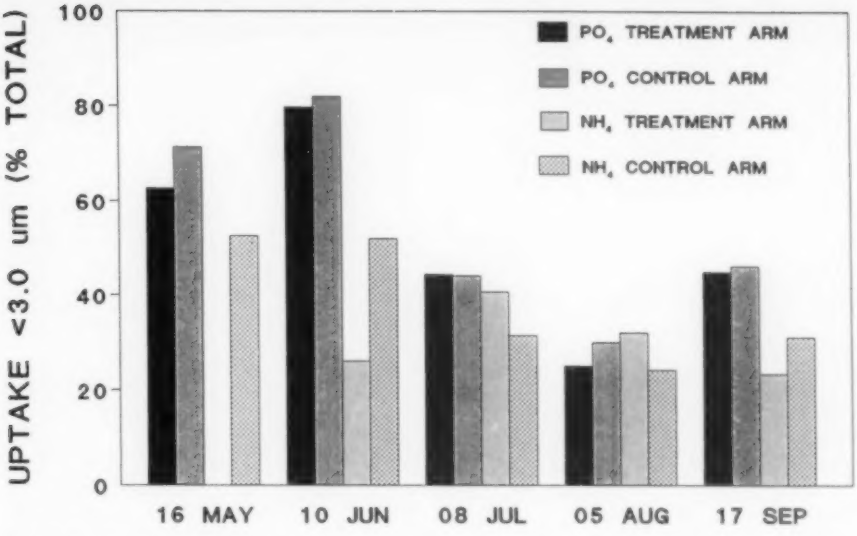

FIG. 4. Percentages of the total phosphate and ammonium fluxes into particulate material that were attributable to the <3 μm size class.

TABLE 3. Proportion of the total ammonium or phosphate uptake in the <3 μm size fraction relative to the chlorophyll in that fraction (i.e. $(V_{<3\ \mu m}/V_{total})/(Chl_{<3\ \mu m}/Chl_{total})$ where V is the uptake rate of phosphate or ammonium and Chl is the concentration of chlorophyll *a*). A value of unity indicates that the nutrient-saturated chlorophyll *a* specific uptake rates for the two size fractions for a given nutrient are the same. A value greater than 1 indicates that a disproportionately greater amount is entering the <3 μm size class based on the chlorophyll *a* in that size fraction.

		Sampling dates				
Nutrient	Treatment	16 May	10 June	8 July	5 Aug.	17 Sept.
NH_4^+	Unfertilized	1.95	1.03	0.73	>0.48[a]	0.47
NH_4^+	Fertilized		1.54	1.05	>0.56[a]	0.27
PO_4^{3-}	Unfertilized	2.64	1.74	1.02	>0.60[a]	0.70
PO_4^{3-}	Fertilized	2.10	2.04	1.14	>0.44[a]	0.54

[a]Chlorophyll *a* concentration in the <3.0 μm size class was below detection limits.

in RURs. The fertilization of one arm of Sproat Lake provided an opportunity to test the time scale and magnitude of the response of the RUR index to changes in N:P supply ratio, caused by a well-defined nutrient addition regime.

There was a lag of several weeks before the increased nutrient load from the relatively N-rich fertilizer (50:1) was reflected by the RUR index (Table 2). After 3 wk of nutrient addition, RURs from both arms of the lake corresponded to an N:P supply ratio of 24:1. Although the actual N:P supply ratio to the phytoplankton was unknown because of other nutrient sources (i.e. regeneration, allochthonous inputs, mixing) the aerial fertilizations occurred during a time of low rainfall and strong stratification and represented an approximate doubling of the annual P load to the lake (J. G. Stockner, unpubl. data). Between June and July, RUR values increased nearly fivefold in the unfertilized arm and almost 20-fold in the fertilized arm, corresponding to estimated N:P supply ratios of 35:1 and 44:1, respectively. These data indicated that fertilization resulted in a higher overall N:P supply ratio, but that this occurred against a background of increasing N:P supply ratio in the lake. The 44:1 N:P supply ratio in the fertilized arm during July was lower than that which was added (50:1), presumably because of dilution by in situ recycling at a lower N:P ratio (34:1, unfertilized arm). Once fertilization stopped, the differences in RURs from the two arms disappeared quickly. Clearly, the increase in RUR caused by fertilization was short-lived once the nutrient inputs had ceased.

RUR-based estimates of N:P supply ratios in Sproat Lake were in the range where the growth rate of some phytoplankton species would be N limited, while others would be P limited (approximately 7:1 to 45:1; see summary in Suttle and Harrison 1988a). In the current study, RURs in the unfertilized arm ranged approximately from 0.5 to 2.5, corresponding to estimated N:P supply ratios of between 24:1 and 34:1 (Table 2). These were greater than the estimates of 18:1 and 24:1 obtained for May 1985 (Suttle and Harrison 1988a), but remained in the range where both N and P would limit phytoplankton growth. Silicate in Sproat Lake remained about 30 μM and was not a factor limiting the growth of diatoms. The differences in RURs potentially could have been caused by changes in the composition or the physiological status of the plankton community.

There was good evidence that changes in community composition were primarily responsible for differences in RURs. For example, the <3 μm size class, which consisted almost entirely of *Synechococcus* (Shortreed and Stockner 1990), generally had higher RUR values than larger cells (Fig. 3), even though both subsets of the community were presumably growing under the same N:P supply ratio. This is consistent with studies showing that *Synechococcus* has about 10-fold higher RURs than some diatoms when both are grown at the same N:P supply ratio (Suttle and Harrison 1988a). Hence, the increase in *Synechococcus* abundance (Fig. 5) likely contributed to the high RUR observed in the fertilized arm in July, and the decline of this species contributed to the subsequent decrease in RUR. Declines in *Synechococcus* populations after cessation of fertilization were also observed in 1985 in Sproat Lake (Shortreed and Stockner 1990) and in nearby Hobiton Lake (Hardy et al.

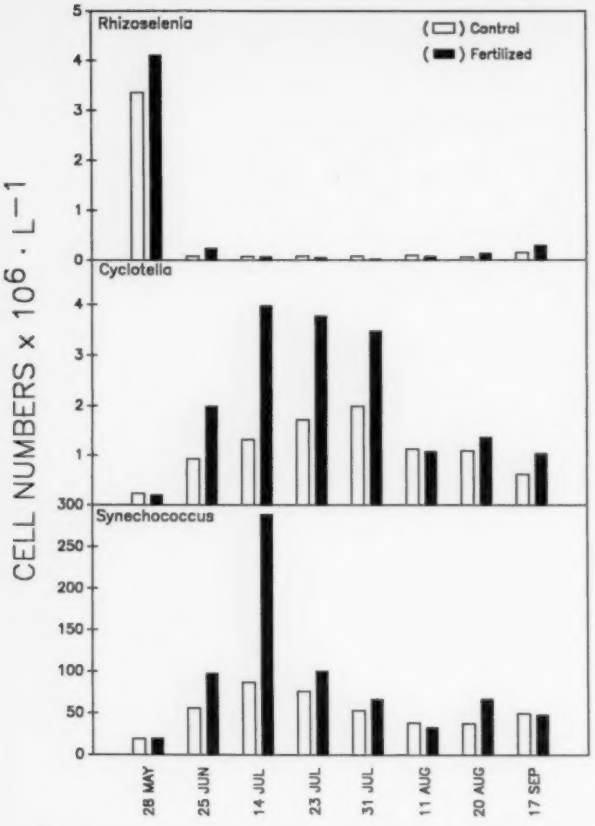

FIG. 5. Effect of lake fertilization on the abundance of the three dominant phytoplankton species in Sproat Lake during the sampling period. *Synechococcus* spp. was the dominant phototroph in the <3 μm fraction, *Rhizoselenia* and *Cyclotella* were the major components of the >3 μm size class.

1986). The 10-fold difference in RURs of different phytoplankton grown at the same N:P supply ratio, the differences in RURs for the two size fractions, and the long lag before RURs responded to fertilization emphasize that changes in RUR were probably mediated by changes in community composition.

RURs are also potentially sensitive to changes in the physiological state of phytoplankton. For example, when grown under phosphate or ammonium limitation, phytoplankton often have much higher maximum uptake rates for the growth-limiting nutrient (e.g. Syrett 1953; Blum 1966; Fuhs et al. 1972; Suttle and Harrison 1986, 1988c). Physiological changes in phytoplankton would occur relatively quickly in response to a changing nutritional environment. As RUR values did not reflect fertilization effects for several weeks after the onset of the nutrient additions, physiological changes in the phytoplankton were probably not driving changes in RUR. In part this may be because RURs are measured over 2-h intervals so that they are relatively insensitive to short-term transients in uptake kinetics.

Size-Dependent Nutrient Uptake and Limitation

In Sproat Lake, cells <3 μm were responsible for about 50% of the total primary productivity (Shortreed and Stockner 1990) and hence would be expected to generate a considerable proportion of the total demand for inorganic nutrients. Previous data from neighbouring oligotrophic Kennedy Lake (Suttle and Harrison 1988b) showed that cells which passed through a 3.0-μm filter accounted for 65–84 and 50–90% of saturated phosphate and ammonium uptake rates, respectively. Our observations for Sproat Lake were similar. The percentage of phosphate uptake that was attributable to organisms passing a 3.0-μm filter ranged from approximately 30% in August to approximately 80% in June and was similar between arms (Fig. 4). The pattern for ammonium uptake was similar to that for phosphate, but was more variable between arms and spanned a smaller range overall (25–53%). With the exception of the fertilized arm in August, the <3 μm fraction accounted for a greater proportion of the saturated phosphate uptake than the saturated ammonium uptake.

In freshwater systems it is not uncommon for N and P to be closely coupled as limiting resources (Dodds et al. 1989), and at many of the estimated N:P supply ratios in Sproat Lake the growth of some phytoplankton species would have been limited by N, while others would have been limited by P. Specifically, the smaller size fraction which consisted almost exclusively of *Synechococcus* (Shortreed and Stockner 1990) should have been N limited because of its high N requirement relative to P (Healey 1985), while the larger diatom-dominated size fraction should have been P limited (Suttle and Harrison 1988a). If this was true, one would expect it to be reflected by the RURs for the two size classes.

RURs were generally greater for the <3 μm fraction than for the >3 μm fraction (Fig. 3). As higher RURs are associated with increasing P deficiency (higher N:P supply ratios), these data seem to indicate that the <3 μm size class was more P limited than the >3 μm fraction. In fact, the results are consistent with the smaller size class having been N limited and the larger P limited. This is because *Synechococcus* can be a poor competitor for ammonium, while diatoms (e.g. *Synedra radians*) can be poor phosphate competitors (Suttle and Harrison 1988a). Even under N limitation, one would expect RURs for the <3 μm fraction to be higher because *Synechococcus* has a low maximum uptake rate for ammonium when N limited, while some diatoms have low maximum phosphate uptake rates when P limited. For example, RURs for the small size fraction in the unfertilized arm (Fig. 3) ranged from about 1.5 to 3.5, similar to values for *Synechococcus* grown at an N:P of 15:1 (Suttle and Harrison 1988a), which should be N limiting for this genus (Healey 1985). Under P limitation (N:P supply ratio of 45:1), RURs for *Synechococcus* were about 60. In contrast, RURs for the >3 μm size class from the unfertilized arm were less than those for the <3 μm fraction (range 0.2–2.3; Fig. 3). In laboratory studies with the diatom *Synedra radians*, ratios >0.1 only occurred under P limitation; N limitation was associated with RURs of 0.03. If RURs determined from laboratory studies can be generalized to *Synechococcus* and the diatoms in Sproat Lake, then the data from the unfertilized arm are consistent with the <3 μm size fraction (*Synechococcus* dominated) being N limited and the >3 μm fraction (diatom dominated) being P limited.

RURs were similar between arms except in the July samples when values for both size classes from the fertilized arm jumped to >9. This is consistent with an increase in N:P supply ratio caused by fertilization and is in the range associated with P limitation in *Synedra radians* and near the transition point

between N and P limitation in *Synechococcus* (Suttle and Harrison 1988a).

As the season progressed the maximum uptake rates of ammonium and phosphate for the <3 μm size class changed from being greater than to being less than the proportion of chlorophyll *a* in that size class (Table 3). A similar trend had been observed between May and September for Kennedy Lake (Suttle and Harrison 1988b). These data suggest that phytoplankton with low chlorophyll *a* to surface area ratios or heterotrophic bacteria were responsible for a considerable proportion of the total uptake of ammonium and phosphate during the early part of the growing season. Previous size-fractionation studies have indicated that bacteria are responsible for a large percentage of the ammonium (Wheeler and Kirchman 1986; Fuhrman et al. 1988) and phosphate flux (Lean and White 1983; Currie and Kalff 1984) into particulate matter when concentrations are low, but at higher concentrations, most of the uptake has been attributed to phytoplankton (Lean and White 1983; Suttle et al. 1990). Hence, it is unlikely that the high chlorophyll *a* specific ammonium and phosphate uptake rates by the <3.0 μm size fraction resulted from a large proportion of the total uptake being associated with bacteria. More likely, the higher rates stemmed from lower chlorophyll *a* per cell in the small size fraction caused by N limitation. As the season progressed, the lower chlorophyll *a* specific uptake rates and the higher proportion of chlorophyll *a* in the <3 μm size class suggest that the phytoplankton biomass was increasingly dominated by small cells that were less nutrient limited than the larger phytoplankton.

Phosphate Turnover

Orthophosphate demand is related to the time it takes for the dissolved pool to turn over. If phosphate is in short supply relative to requirements for growth, the flux through the dissolved pool will be rapid and the turnover time short. In contrast, when phosphate is in excess of growth requirements, the pool cycles slowly. The dissolved phosphate turnover times in Sproat Lake were on the order of minutes (Fig. 2) and in the range where phosphate was potentially in short supply (Lean et al. 1983). By July the fertilizer additions resulted in about a threefold increase in chlorophyll *a* compared with the unfertilized arm (Tables 1 and 2). Yet, because of the high N:P ratio of the added nutrients the increased phosphate load did not result in longer phosphate turnover times. In fact, the dissolved phosphate pool continued to cycle more quickly in the fertilized arm than in the unfertilized arm for the remainder of the study.

The results of this study are consistent with RUR being sensitive to the N:P supply ratio. However, in Sproat Lake there was a considerable lag before changes in N:P supply ratio were reflected by RUR values. Consequently, RURs may not be appropriate for assaying short-term (days to weeks) changes in N:P supply ratio, but should reflect the nutrient regime in lakes over longer intervals (months). Further studies are needed on a larger suite of lakes to ascertain the general utility of using ammonium and phosphate kinetics data as indicators of in situ N:P supply ratios.

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Effects of Rosyside Dace (*Clinostomus funduloides*) on Microhabitat Use of Rainbow Trout (*Oncorhynchus mykiss*)

Gary D. Grossman and Vital Boulé

School of Forest Resources, University of Georgia, Athens, GA 30602, USA

Grossman, G. D., and V. Boulé. 1991. Effects of rosyside dace (*Clinostomus funduloides*) on microhabitat use of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1235–1243.

We experimentally examined the effects of competition for space between rainbow trout (*Oncorhynchus mykiss*) and rosyside dace (*Clinostomus funduloides*) in an artificial stream. These species generally do not exhibit statistically significant differences in microhabitat use in Coweeta Creek, North Carolina, USA. Two competition (trout and dace) and two control (trout only) trials were conducted during spring and summer. Microhabitat use data indicated that the presence of dace did not produce microhabitat shifts in trout. Most behavioral changes could be attributed to acclimation effects or intraspecific competition. Based on field and laboratory results, it does not appear that interspecific competition with dace strongly affects the use of spatial resources in rainbow trout.

Nous avons examiné de façon expérimentale les effets de la concurrence spatiale entre la truite arc-en-ciel (*Oncorhynchus mykiss*) et le méné (*Clinostomus funduloides*) dans un cours d'eau artificiel. Ces espèces ne présentent généralement pas de différences statistiquement significatives dans l'utilisation du microhabitat dans le ruisseau Coweeta, en Caroline du Nord (États-Unis). Des essais ont été menés au printemps et en été, deux en situation de concurrence (truite et méné) et deux essais témoins (truite seulement). Les données sur l'utilisation du microhabitat ont montré que la présence du méné ne causait pas de modification à cet égard chez la truite. La plupart des changements comportementaux pouvaient être attribués aux effets de l'acclimatation ou à la concurrence intraspécifique. Selon les résultats obtenus sur le terrain et en laboratoire. Il ne semble pas que la concurrence interspécifique avec le méné ait un effet important sur l'utilisation des ressources spatiales chez la truite arc-en-ciel.

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Competition for space or spatially linked trophic resources occurs in both marine (Hixon 1980; Larson 1980; Ebersole 1985) and lacustrine fish assemblages (Werner and Hall 1977, 1979). This phenomenon also has been observed among stream fishes (Cadwallader 1975; Fausch and White 1981; Baltz et al. 1982; Greenberg 1988), although the frequency of competition for space in stream fish assemblages is subject to debate. Some researchers suggest that partitioning of space is important for coexistence among stream fishes (Mendelson 1975; Gorman and Karr 1978; Page and Schemske 1978; Herbold 1984; Moyle and Vondracek 1985) whereas others maintain that many differences in habitat use can be attributed to noninteractive processes such as evolutionary constraints on behavior and physiology (Angermeier 1987; Ross et al. 1987; Grossman and Freeman 1987; Grossman et al. 1987a, 1987b). Although both field and experimental data will be required to assess the validity of these viewpoints, such combined studies of competition are uncommon.

Species

Rainbow trout (*Oncorhynchus mykiss*) is an aggressive, territorial, stream-dwelling salmonid. Although native to the northern Pacific Rim, it has been widely introduced, and is now found on at least six continents (MacCrimmon 1971). Self-sustaining populations of this species are widely distributed throughout eastern North America (MacCrimmon 1971; Lee et al. 1980) where they were introduced in the late nineteenth century. In Coweeta Creek, North Carolina, USA, rainbow trout are territorial and preferentially occupy water column microhabitats. These microhabitats are located in deeper water, far from shelter, and possess moderate to high focal point velocities (Grossman and Freeman 1987). Microhabitat use by trout is similar to that of several sympatric minnows, in particular the rosyside dace (*Clinostomus funduloides*). Although dace tend to occur over slightly greater amounts of erosional substrate (i.e. cobble, boulder, etc.), Grossman and Freeman (1987) demonstrated that the microhabitats occupied by trout and dace in Coweeta Creek generally were not statistically distinguishable, and this conclusion has been supported by five additional years of microhabitat observations (G. Grossman et al., unpubl. data). In addition, most dace (i.e. 80%) do not defend feeding territories (Freeman 1990). Based on field data, Grossman and Freeman (1987) suggested that competition for space was unlikely between these species. Nontheless, these descriptive data cannot be used to reject the competition hypothesis without ambiguity. Given this fact, we decided to conduct a series of competition experiments for space in an artificial stream. Such a study was further warranted by the almost complete lack of studies of fish competition that include both long-term descriptive and experimental data. The questions addressed in our work include (1) does the presence of dace produce niche shifts in trout and (2) are behavioral interactions between dace and trout stronger than trout–trout interactions.

Methods

Stream Channel

We conducted trials in a 10-m, oval, artificial stream constructed of fiberglassed plywood and containing Plexiglas viewing ports. Width of the stream varied from about 50 to 75 cm, depending on the position in the oval. The stream was continuously fed from a reservoir containing chilled deionized tap water. Flow rates were sufficient to completely replace the water in the stream every 5 d.

The channel contained pools at each end, separated by riffles. Riffles had gently inclined fore- and back-slopes, which simulated run microhabitats. Depth in the channel ranged from 32 cm (pools) to 5 cm (riffles) and fishes moved freely across riffles. We generated currents up to 65 cm/s using pumps located at both ends of the stream. Electronically controlled photoperiods of 13 h light : 11 h dark (spring) and 14.5 h light : 9.5 h dark (summer) were used. A 1-h phase of increasing or decreasing illumination (i.e. dawn and dusk) separated light and dark periods. Water temperature for spring trials was 10°C whereas we conducted summer trials at 15°C. Spring and summer trials began respectively in April and August and ended in August and December. Each set of seasonal data consisted of two interspecific competition trials (i.e. experiments with trout and dace, henceforth competition) and two control trials (experiments with trout only, henceforth control). We collected wild fishes from the Coweeta Creek drainage between March and August (spring trials) and July and November (summer trials). Because we had only one experimental channel, it was not possible to complete all trials during their respective seasons (i.e. all summer trials between 21 June and 22 September).

To ascertain the suitability of the channel as a habitat, we compared microhabitat availability data collected at the beginning ($n = 40$) and end ($n = 40$) of each trial (total $n = 320$ per season) with those collected seasonally in Coweeta Creek (spring 1984 and 1988, and summer 1983, 1984, 1986, 1988, and 1989 (see Grossman and Freeman 1987). Availability data included the following measurements: (1) depth, (2) average velocity, and (3) the percentage of bedrock (= exposed plywood bottom), boulder, cobble, gravel, sand, silt, and debris in the substrate. Data were taken in randonly chosen 20×20 cm^2 quadrats in both the channel and Coweeta Creek. Methodological details are presented in Grossman and Freeman (1987) and Grossman et al. (1987a).

Availability comparisons indicated that there were no significant differences for any parameter within the 17-d observation period constituting a trial (see Experimental Procedures, Kruskall–Wallis test, all p's > 0.05). In addition, a comparison of availability data among all trials yielded only two significant differences (average velocity in one control trial was 14 versus 10 cm/s in a second control trial; the same was true for sand, 13 versus 11%) out of 64 tests. These differences were small, however, and within the number expected by chance alone (i.e. three significant tests). Hence, we pooled data from both spring and summer trials for channel versus Coweeta Creek comparisons. These comparisons (Mann–Whitney U test, all p's < 0.05) indicated that the channel had lower velocities, fewer boulders, less rubble, silt, and debris, and more gravel and bedrock (i.e. exposed plywood bottom) than Coweeta Creek. Nevertheless, the values of channel microhabitat parameters still were well within the statistical range of environmental measurements recorded for our study site in Coweeta Creek.

We also evaluated the suitability of the channel as a habitat for trout and dace by monitoring mortality and testing for significant differences in mean length and mass during trials (Mann–Whitney U test).

Experimental Procedures

We began trials by placing 10 trout in the channel. This yielded a density of approximately 1 trout/m^2, a density observed in high-quality habitats in Coweeta Creek (G. Grossman, pers. obs.). Rosyside dace were used as potential competitors in trials. Mean standard lengths ($\pm$ SD) of trout and dace used in trials ranged from 39 ± 2 to 77 ± 2 mm depending on season. Trout were all 0+ in age and dace ranged from approximately 1+ to 3+. We used dace of equal or slightly larger size than trout because dominance in trout is size-linked, with larger specimens typically dominant over smaller fish, regardless of species (Li 1975; Noakes 1980; Fausch 1988). Given this fact, it seemed unlikely that smaller dace would be capable of dominating larger trout. Hence, our decision to conduct trials in which the size advantage was equal or reversed. This decision also limited competition trials to spring and summer because these are the only seasons in which dace in Coweeta Creek are equal to or larger than trout (G. Grossman et al., unpubl. data).

We fed fishes trout chow (1.18–2.00 mm, maximum diameter) with an automatic feeder at a rate of 2–3 pellets/min. Food was released from a single location at the channel's surface to simulate invertebrate drift. Current speeds were sufficient to maintain many pellets in the water column, even after they had absorbed some water. Trout and dace occupied microhabitats throughout the channel and did not concentrate directly below the point of food release. Both species fed on pellets from the surface and the water column, foraging behavior typical of wild fish (Hill 1989; G. Grossman, unpubl. data).

We initiated observations after allowing fishes to acclimate for 1–2 d. Our design precluded an acclimation period for dace in competition trials (see Statistical Design). Observations were made twice daily (spring: between 08:00 and 11:00 and 15:00 and 18:00; summer: between 07:30 and 10:30 and 16:00 and 19:00) to control for diurnal changes in activity. We controlled for changes in light intensity by initiating and terminating observations no sooner than either 0.5 h after dawn or before dusk. Observations were not made at night because both species generally are inactive during this period in Coweeta Creek (Hill 1989).

During observation periods, we scanned the water column and substrate through viewing ports in a black plastic shroud covering the sides of the channel. When an undisturbed fish was located, we recorded the following data: (1) fish length (nearest millimetre, estimated visually), (2) longitudinal position (nearest centimetre, X coordinate), (3) distance from the center of the channel (nearest centimetre, Y coordinate), (4) distance from the substrate (nearest centimetre, Z coordinate), (5) distance from shelter (i.e. an object large enough to conceal 50% of the fish's body), and (6) microhabitat type (pool, run, or riffle). Linear measurements were verified either through comparisons with objects of known size (i.e. cobbles) or with a centimetre scale located on the outside of the channel (longitudinal position only). After these data were collected, we made 3 min of behavioral observations on each fish, recording

the following information: (1) number of strikes at food particles, (2) reaction distance to food particles, (3) number of seconds spent within 15 cm of a conspecific, (4) number of seconds spent within 15 cm of a heterospecific, (5) number of chases by the focal individual of a conspecific (Hartman 1965), (6) number of chases by the focal individual of a heterospecific, (7) number of chases of the focal individual by a conspecific, and (8) number of chases of the focal individual by a heterospecific. When the entire channel had been examined, we returned to the position of each fish (based on its X, Y, and Z coordinates) and obtained the following data: (1) focal point velocity, (2) average velocity, (3) distance from shelter, (4) shelter size, and (5) the percent composition of bedrock, boulder, cobble, gravel, sand, and debris in a 20×20 cm^2 quadrat directly underneath the fish. Measurement techniques are described in Grossman and Freeman (1987) and Grossman et al. (1987a).

Statistical Design

A test for interspecific competition for space between trout and dace required both control and competition trials. In order to separate the effects of acclimation, intraspecific competition, and interspecific competition, it was necessary to partition each trial into three sequential segments. These segments differed between trials because control and competition trials examined different aspects of the design. Furthermore, the separation of all potential effects required comparisons of shifts both within trials (i.e. the three sequential segments) and between trials (i.e. control trials versus competition trials). The logical structure of our design is presented below.

Control trials consisted of 5 d of observations on 10 trout (full density 1 segment); five trout were then removed. These trout were representative of the size ranges used in the trial. We then completed a second 5-d period of measurements (half density segment, total density = 5), replaced the previously removed trout (total density = 10), and conducted a final 5 d of observations (full density 2 segment). Competition trials were identical except that the five trout removed during the second 5 d of observations were replaced by five dace (competition segment (=treatment), total density = 10). For simplicity, both half-density and competition segments of control and competition trials are referred to as treatment segments. This design held intraspecific competition constant in comparisons of results of control and competition trials (see Table 1) and enabled us to test for a variety of acclimation, intraspecific competitive, and interspecific competitive effects. For example, if dace did not affect microhabitat use by trout, then only intraspecific competition was present. This would produce identical results for both control and competition trials, with full density segments 1 and 2 differing significantly from treatment segments (Table 1). Conversely, if only interspecific competition were present, then there would be no significant differences among segments of control trials, but the treatment segment of competition trials would be significantly different from both full density segments (Table 1). Table 1 presents a variety of more complex effects that were detectable using this design (e.g. presence of both inter- and intraspecific competition). However, these effects were not observed, and therefore are not described.

To quantify patterns of nonrandom microhabitat use, we employed the principal component (PCA) technique of Grossman and Freeman (1987) and Grossman et al. (1987a). In brief, we performed separate PCA's (correlation matrix solution) on the habitat availability data for each trial. Data were transformed by either arcsine (substrate data) or ln (linear measures). We only interpreted axes that had both eigenvalues greater than 1 and significant values for Barlett's sphericity test. We then multiplied the fish microhabitat data by the scoring coefficient matrix of the habitat availability PCA. This yielded a score for each fish on each axis and had the effect of superimposing the microhabitat use data on the total available habitat space. We then compiled frequency distributions for both microhabitat availability and microhabitat use data and tested for significant differences in these distributions using a partitioned chi-square test for independence (Zar 1984). The partitioned analysis enabled us to determine microhabitat categories in which fishes were significantly over- and underrepresented. Alpha levels were adjusted for multiple comparisons using the Dunn–Sidak procedure (Ury 1976).

We tested for microhabitat shifts within trials by conducting Kruskal–Wallis tests on the mean component scores for fishes in each trial segment (i.e. full density 1 versus treatment versus full density 2). If a significant difference was obtained, pairwise differences were identified using Tukey–Kramer tests. Because microhabitat availability did not differ significantly among trials, we based competition and control comparisons on microhabitat use data alone; comparisons of microhabitat use and availability (i.e. nonrandom use data) are presented briefly in the text (further details are available from the senior author on request). Behavioral data also were examined using Kruskal–Wallis and Tukey–Kramer tests. Statistical calculations were made using SAS (SAS Institute, Inc. 1982) or custom written programs (i.e. partitioned chi-square analysis and sphericity test).

Results

Suitability of the Experimental Channel

The channel provided an adequate experimental habitat for trout because mortality was low (1%), and there were no significant differences in mean length during trials (Table 2). We obtained a similar result for mass data although there was a significant decrease in mean body mass during the control 1 trial for spring (Table 2). Visual comparisons of behavior of both trout and dace in the channel and Coweeta Creek also indicated similar behavioral repertoires and levels of interaction (G. Grossman, pers. obs.; J. Hill, pers. comm.). Hence, although differences existed between the stream channel and Coweeta Creek, these differences did not appear to drastically alter the behavior, growth, or mortality of specimens.

Microhabitat Use

The PCA produced up to four significant components which explained between 63 and 78% of the variance in microhabitat use for each trial. The major microhabitat availability gradients elucidated by the PCA contrasted shallow, high-velocity areas with erosional substrates (riffles) against deeper, low-velocity locations with depositional substrates (pools).

In both spring and summer trials, trout generally avoided shallow, high-velocity microhabitats with large amounts of gravel and bedrock. When a preference for substrate was exhibited, it typically was a mixed substrate type (cobble, gravel, sand, and organic debris), and boulders and bedrock alone almost always were avoided. Trout also avoided microhabitats

TABLE 1. Representation of the potential and actual (see Tables 3–5) effects detectable using our experimental design. Trial refers to a single 17-d experiment whereas segment refers to the three 5-d observation periods within each trial. Effects were assessed both by sequential comparisons within a trial (e.g. FD1 vs. T vs. FD2) as well as by comparison of results between trials (i.e. control vs. competition trials). FD1 = full density 1 segment, FD2 = full density 2 segment, and T = treatment.

		Control trials (FD1 = 10 trout, T = 5 trout removed, FD2 = 10 trout)		Competition trials (FD1 = 10 trout, T = 5 trout + 5 dace, FD2 = 10 trout)	
Effect	Abbreviation	Result	No. of occurrences (Tables 3–5)	Result	No. of occurrences (Tables 3–5)
None	N	FD1 = T FD1 = FD2 FD2 = T	26	FD1 = T FD1 = FD2 FD2 = T	19
Simple acclimation (1st segment)[a]	A	FD1 ≠ T FD1 ≠ FD2 FD2 = T	2	FD1 ≠ T FD1 ≠ FD2 FD2 = T	1
Simple acclimation (1st two segments)	A	FD1 = T FD1 ≠ FD2 FD2 ≠ T	4	FD1 = T FD1 ≠ FD2 FD2 ≠ T	5
Complex acclimation	A	FD1 = T FD1 ≠ FD2 FD2 = T	1	FD1 = T FD1 ≠ FD2 FD2 = T	4
Complex acclimation	A	FD1 = T FD1 = FD2 FD2 ≠ T	3	FD1 = T FD1 = FD2 FD2 ≠ T	2
Complex acclimation	A	FD1 ≠ T FD1 = FD2 FD2 = T	1	FD1 ≠ T FD1 = FD2 FD2 = T	3
Intraspecific competition only	I	FD1 ≠ T FD1 = FD2 FD2 ≠ T	3	FD1 ≠ T FD1 = FD2 FD2 ≠ T	1
Interspecific competition only	C	FD1 = T FD1 = FD2 FD2 = T	0	FD1 ≠ T FD1 = FD2 FD2 ≠ T	2
Total density (i.e. interspecific competition = intra specific competition)	D	FD1 ≠ T FD1 = FD2 FD2 ≠ T	0	FD1 = T FD1 = FD2 FD2 = T	0
Both intraspecific and interspecific competition present; intraspecific competition > interspecific competition[b]	IS	FD1 ≠ T FD1 = FD2 FD2 ≠ T	0	FD1 > T FD1 = FD2 FD2 > T	0
Both intraspecific and interspecific competition present; interspecific competition > intraspecific competition[b]	CS	FD1 ≠ T Fd1 = FD2 FD2 ≠ T	0	FD1 < T FD1 = FD2 FD2 < T	0

[a]It is possible that this response represents a competition effect plus an experience effect in which the competitor effect lingered into the second full density segment.

[b]For both of these cases the shift in the treatment segment of the control experiments should be significantly less than the shift manifested in the treatment segment of competition experiments. This result was obtained because only weak intraspecific competition is present in the treatment segment of control trials.

close to the edge of the artificial stream which lacked sand and debris. Conversely, in several control trials (2, 3, and 4), trout preferred edge microhabitats with substantial amounts of debris. Trout also were occasionally overrepresented in slow, deep microhabitats.

Dace preferred deeper microhabitats with lower velocities and more heterogeneous substrates than trout. Dace also were overrepresented closer to the edge of the channel during summer trials. Finally, dace avoided deep microhabitats close to the center of the stream during one spring trial.

Effects of Dace on Trout

Microhabitat use

The presence of dace did not produce strong microhabitat shifts in trout (Table 3; Fig. 1). Only 4 of 29 tests comparing

TABLE 2. Changes in mean length and mass for trout in all trials. Statistical comparisons were made using Mann–Whitney U tests with $\alpha = 0.05$. Sample sizes equal 10 except where otherwise noted. Mean (± SD) lengths (mm) of dace used in competition trials are as follows: (1) spring competition 1: 65 ± 2, (2) spring competition 2: 62 ± 3, (3) summer competition 1: 65 ± 5, (4) summer competition 2: 65 ± 6. $^*p < 0.05$.

	Spring trials				Summer trials			
	Control 1	Control 2	Competition 1	Competition 2	Control 1	Control 2	Competition 1	Competition 2
Pretrial mean (± SD) length (mm)	77 ± 2	67 ± 6	39 ± 2	51 ± 6	59 ± 4	76 ± 6	56 ± 7	57 ± 4
Postrial mean (± SD) length (mm)	76 ± 3	69 ± 6	40 ± 3	53 ± 5[a]	61 ± 5	76 ± 7	58 ± 6	58 ± 4
Pretrial mean (± SD) mass (g)	6.7 ± 1.0	4.4 ± 1.1	0.8 ± 0.2	1.9 ± 0.7	3.1 ± 0.6	5.1 ± 1.4	2.7 ± 0.8	2.7 ± 0.5
Posttrial mean (± SD) mass (g)	5.7 ± 0.8*	4.3 ± 1.2	0.8 ± 0.2	2.0 ± 0.6[a]	3.4 ± 0.9	5.8 ± 1.6	2.8 ± 0.9[a]	2.9 ± 0.8[b]

[a] $n = 8$; two trout jumped out of the holding tank during treatment segments of competition trials.
[b] $n = 9$; one trout jumped out of the holding tank.

TABLE 3. Test for the presence of competitively induced shifts in microhabitat use between trout and dace. A Kruskal–Wallis test was used to test for significant differences in trout PCA scores among segments (i.e. full density 1, treatment, full density 2) within a trial. $^*p < 0.05$; other components were not significant. For ease of interpretation, compare these results with those predicted by Table 1.

	Component, spring trials		Component, summer trials	
Control 1	1	FD1 = T	1	
	2*	FD1 ≠ FD2 A FD2 ≠ T	2	
	3		3	
	4		4	
Control 2	1*	FD1 ≠ T A FD1 ≠ FD2 FD2 = T	1	
	2		2	
	3		3	
	4		4	
Competition 1	1		1*	FD1 ≠ T A FD1 = FD2 FD2 = T
	2		2	
	3		3	
			4	
Competition 2	1		1	
	2		2	
			3	
			4*	FD1 ≠ T A FD1 = FD2 FD2 = T

trout PCA scores within trials yielded significant results. Two shifts occurred in competition trials, and two were observed in control trials. A graphical examination (Fig. 1) of significant shifts suggests that some of these effects may have been caused by chance or sample size differences (trout densities in treatment segments of all trials were 50% of full density segments). In addition, the observed shifts were not consistent with the expected patterns of intra- or interspecific competitive effects (Table 1). All four shifts were indicative of acclimation effects.

Behavioral effects

Behavioral analyses examined the effects of dace on food aquisition and aggression (Tables 4 and 5). Forty-two percent (i.e. 20) of the comparisons did not exhibit effects of simple intra- or interspecific competition, total density, or acclimation (Tables 1, 4 and 5). The only variable that always exhibited a response was the percentage of time a focal individual spent with a conspecific within 15 cm (Tables 4 and 5). This parameter displayed the only intraspecific density response observed ($n = 4$, 8% of total comparisons). An additional 46% ($n = 22$) of tests exhibited acclimation effects, although this rarely was the simple pattern portrayed in Table 1. In addition, acclimation effects were much more common in summer ($n = 18$) versus spring ($n = 4$) trials. Most acclimation effects involved a difference in just one trial segment (e.g. mean distance moved for food pellets — spring competition trial 1 and summer competition trial 1 — full density 1 = treatment, full density 2 = treatment, full density 2 ≠ (greater) full density 1). Acclimation effects were particularly pronounced because most parameters involved feeding and intraspecific interactions (Tables 4 and 5). We only observed an interspecific competition effect in one variable, distance from shelter, during summer competition trials (Tables 1, 4, and 5). Nonetheless, even this result was inconsistent. In one trial, trout were further from shelter in the presence of dace whereas in the remaining trial, they were closer to shelter (Table 5). Additionally, one of the control trials displayed an acclimation effect for this variable. Interspecific competitor effects were not apparent during spring competition trials (Table 4).

It is also possible that either an acclimation effect or a competitor plus experience effect may have occurred in the number of food pellets captured per minute during spring trial 1 (control trial 1 = no effect, competition trial 1 = full density 1 ≠ (less) full density 2, treatment; full density 2 = treatment). Once again, given the lack of competitor responses in other behavioral and microhabitat parameters, it seems more likely that this response actually was an acclimation effect.

Comparisons of both intra- and interspecific aggression rates (i.e. chases of and by conspecifics and heterospecifics) indicated that trout were more aggressive towards conspecifics than towards dace. In all four experiments, rates of intraspecific aggression in the full density 2 segments were significantly higher than rates of interspecific aggression during treatment segments (t-tests adjusted for multiple comparisons, all p's < 0.005). In fact, we only observed chases of trout by dace in one of four trials. Consequently, there was little evidence that interspecific aggression had a stronger effect on microhabitat use than intraspecific aggression.

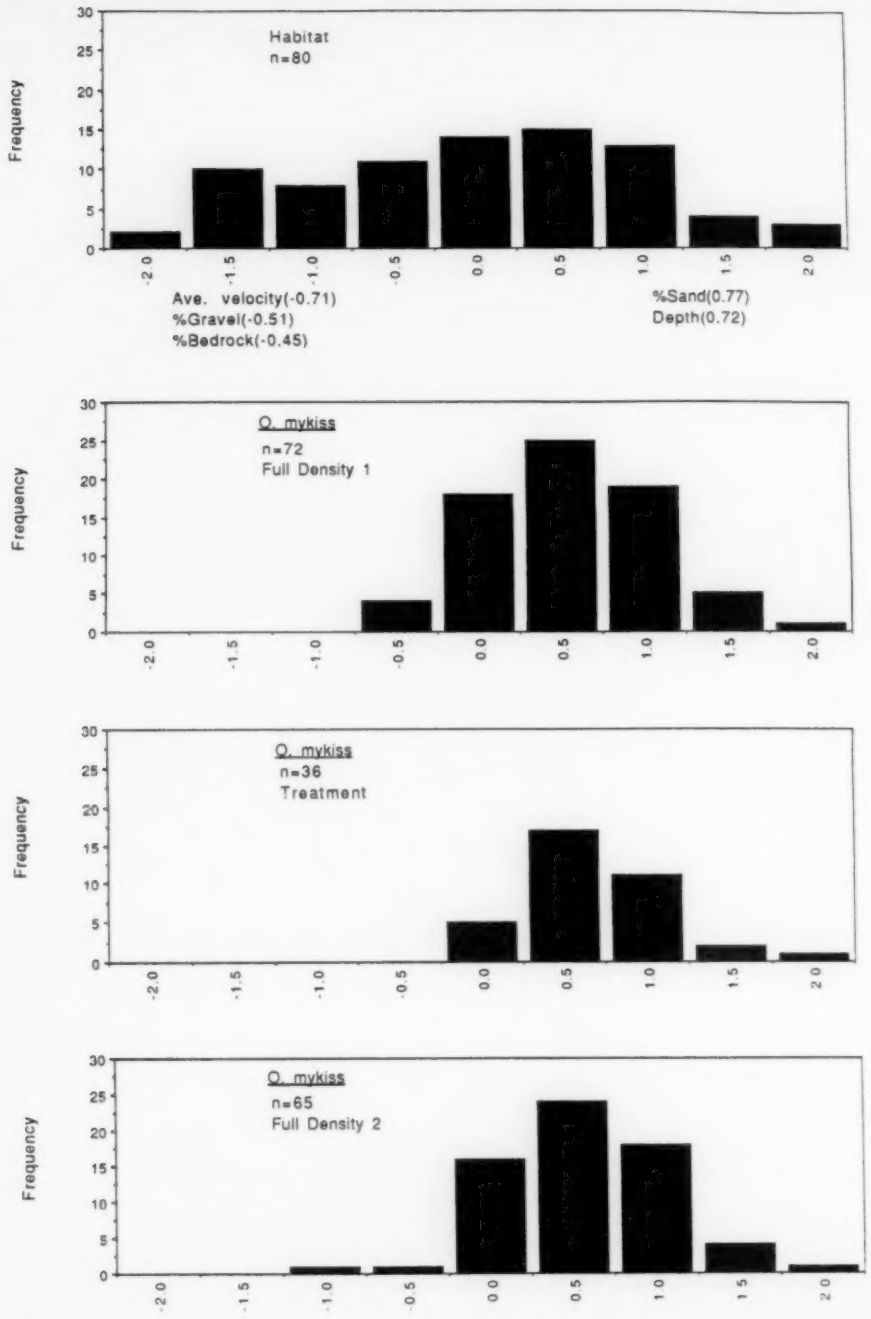

FIG. 1. Example of the component score distributions for both microhabitat availability and use data for trout (summer competition trial 1, component 1). In this example, Kruskal–Wallis and Tukey–Kramer tests on component score distributions indicated that full density 1 score distributions were significantly different from those of the treatment segment. There were no significant differences between score distributions of (1) full density 1 and full density 2 and (2) full density 2 and the treatment. This result represents a complex acclimation effect described in Table 1. See the text for a more thorough explanation.

TABLE 4. Behavioral data (means ± SD) for trout in spring trials. Control and treatment segments of trials are abbreviated as follows: FD1 = full density 1 segment, T = treatment segment (either half density segment, control trials or competition segment, competition trials), and FD2 = full density 2 segment. Effect designations are as follows: N = no effect, I = intraspecific competition effect, C = interspecific competition effect, A = acclimation effect (see Table 1). Segments followed by the same letter are not significantly different. Note that statistical results only apply to sequential comparisons with a trial. Kruskal–Wallis test, $\alpha = 0.05$, with Tukey–Kramer a posteriori tests.

Variable	Segment	Control trial 1	Effect	Control trial 2	Effect	Competition trial 1	Effect	Competition trial 2	Effect
Distance from shelter (cm)	FD1	7.5 ± 6.2 ab	FD1 = T A	4.4 ± 3.3 a	FD1 = T N	8.3 ± 6.2 a	FD1 = T N	6.5 ± 4.4 a	FD1 = T N
	T	6.8 ± 6.6 a	FD1 = FD2	2.7 ± 3.5 a	FD1 = FD2	9.1 ± 7.3 a	FD1 = FD2	5.0 ± 4.9 a	FD1 = FD2
	FD2	9.7 ± 6.1 b	FD2 ≠ T	4.8 ± 4.1 a	FD2 = T	8.7 ± 7.1 a	FD2 = T	6.4 ± 5.1 a	FD2 = T
No. of food pellets captured/min	FD1	0.5 ± 0.7 a	FD1 = T N	0.3 ± 0.4 a	FD1 = T N	0.8 ± 0.6 a	FD1 ≠ T A*	1.5 ± 1.5 a	FD1 = T N
	T	0.6 ± 0.5 a	FD1 = FD2	0.5 ± 0.7 a	FD1 = FD2	2.0 ± 1.0 b	FD1 ≠ FD2	1.4 ± 1.3 a	FD1 = FD2
	FD2	0.6 ± 0.5 a	FD2 = T	0.6 ± 0.7 a	FD2 = T	2.0 ± 1.0 b	FD2 = T	1.6 ± 1.2 a	FD2 = T
Mean distance moved to capture food	FD1	29 ± 26 a	FD1 = T N	33 ± 62 a	FD1 = T N	34 ± 28 a	FD1 = T A	52 ± 41 a	FD1 = T N
	T	41 ± 37 a	FD1 = FD2	34 ± 78 a	FD1 = FD2	42 ± 26 ab	FD1 ≠ FD2	52 ± 42 a	FD1 = FD2
	FD2	21 ± 17 a	FD2 = T	39 ± 48 a	FD2 = T	45 ± 29 b	FD2 = T	43 ± 28 a	FD2 = T
Chases of conspecifics/min	FD1	0.14 ± 0.41 a	FD1 = T N	0.04 ± 0.17 a	FD1 = T N	0.07 ± 0.26 a	FD1 = T A	0.23 ± 0.80 a	FD1 = T N
	T	0.08 ± 0.27 a	FD1 = FD2	0.10 ± 0.49 a	FD1 = FD2	0.17 ± 0.52 a	FD1 ≠ FD2	0.03 ± 0.12 a	FD1 = FD2
	FD2	0.20 ± 0.60 a	FD2 = T	0.04 ± 0.17 a	FD2 = T	0.30 ± 0.51 b	FD2 ≠ T	0.20 ± 0.61 a	FD2 = T
Chases of heterospecifics/min	FD1	—		—		—		—	
	T	—		—		0.0 ± 0.0		0.0 ± 0.0	
	FD2	—		—		—		—	
Chases of focal individual by a conspecific/min	FD1	0.09 ± 0.32 a	FD1 = T N	0.01 ± 0.08 a	FD1 = T N	0.06 ± 0.17 a	FD1 = T N	0.03 ± 0.14 a	FD1 = T N
	T	0.01 ± 0.06 a	FD1 = FD2	0.00 ± 0.00 a	FD1 = FD2	0.13 ± 0.40 a	FD1 = FD2	0.02 ± 0.08 a	FD1 = FD2
	FD2	0.04 ± 0.14 a	FD2 = T	0.04 ± 0.13 a	FD2 = T	0.14 ± 0.32 a	FD2 = T	0.12 ± 0.37 a	FD2 = T
Chases of focal individual by a heterospecific/min	FD1	—		—		—		—	
	T	—		—		0.0 ± 0.0		0.0 ± 0.0	
	FD2	—		—		—		—	
% Time spent by trout with a conspecific within 15 cm	FD1	31 ± 44 a	FD1 ≠ T I	13 ± 31 a	FD1 ≠ T I	14 ± 20 ab	FD1 = T A	31 ± 44 a	FD1 ≠ T I
	T	9 ± 25 b	FD1 = FD2	0 ± 1 b	FD1 = FD2	10 ± 26 a	FD1 = FD2	9 ± 25 b	FD1 = FD2
	FD2	33 ± 37 a	FD2 ≠ T	13 ± 27 a	FD2 ≠ T	20 ± 25 b	FD2 ≠ T	33 ± 37 a	FD2 ≠ T
% time spent with a heterospecific within 15 cm	FD1	—		—		—		—	
	T	—		—		3 ± 18		0.0 ± 0.0	
	FD2	—		—		—		—	

*This result may also have been produced by a competitor plus experience which lingered into the second control segment.

Discussion

Our results provide little support for the hypothesis that dace produce shifts in microhabitat use by trout. This statement cannot be left unqualified, however, because experiments were conducted in an artificial stream, under nonlimiting food conditions. Nonetheless, the values of microhabitat parameters within the stream environment were well within the statistical range of physicochemical conditions experienced by trout and dace in Coweeta Creek. These findings, combined with the observational data of Grossman and Freeman (1987), suggest that dace do not compete for space with trout in Coweeta Creek.

Our conclusions also are supported by the work of Hill (1989) who developed an intraspecific bioenergetic model of microhabitat selection for trout and dace. Although this model does not include interspecific processes, its results, combined with those of the present study, yield a more complete understanding of the trout–dace interaction. Hill (1989) found that microhabitat use in trout and dace is strongly linked to the respective abilities of these species to capture food at a given velocity. When energetic costs are included in this model, they contribute minimally to its predictive ability; the dominant factor is the relationship between prey capture and velocity (Hill 1989). Energetic costs are not unrelated to microhabitat use of trout and dace in Coweeta Creek, however. In fact, both dace and trout may avoid velocities at which energetic expenditures are high (Facey 1987). Hill (1989) found, however, that decreases in prey capture success occurred at velocities below which energetic constraints became important (Facey 1987). Hence, capture success is more important than energetic constraints in determining microhabitat use. Finally, because trout and dace in Coweeta Creek occupy velocities close to their different energetic optima, Hill (1989) suggested that interspecific interference competition between these species may be unimportant.

Other investigators have used artificial streams to quantify interactions between trout and nonsalmonid stream fishes. Li (1975) and Symons (1976), respectively, found that rainbow trout and Atlantic salmon (*Salmo salar*) dominated various minnows (Cyprinidae) and suckers (Catostomidae) in experimental trials. There was little indication that the nonsalmonid species affected microhabitat use in these salmonids, nor was there strong evidence that the energy expended by salmonids in interspecific interactions was significant. In both systems, intraspecific interactions among salmonids were far more frequent than interspecific conflicts. Although neither Li (1975) nor Symons (1976) verified their results with microhabitat data from natural habitats, their results are similar to our finding.

TABLE 5. Behavioral data (means ± SD) for trout in summer trials. Control and treatment segments of trials are abbreviated as follows: FD1 = full density 1 segment, T = treatment segment (either half density segment, control trials or competition segment, competition trials), and FD2 = full density 2 segment. Effect designations are as follows: N = no effect, I = intraspecific competition effect, C = interspecific competition effect, A = acclimation effect (see Table 1). Segments followed by the same letter are not significantly different. Note that statistical results only apply to sequential comparisons with a trial. Kruskal–Wallis test, $\alpha = 0.05$, with Tukey–Kramer a posteriori tests.

Variable	Segment	Control trial 1	Effect	Control trial 2	Effect	Competition trial 1	Effect	Competition trial 2	Effect
Distance from shelter (cm)	FD1	4.1 ± 4.2 a	FD1 = T N	4.0 ± 2.3 a	FD1 ≠ T A	3.7 ± 3.2 a	FD1 ≠ T C	3.5 ± 3.4 a	FD1 ≠ T C
	T	3.7 ± 2.6 a	FD1 = FD2	3.1 ± 2.4 b	FD1 = FD2	7.4 ± 5.3 b	FD1 = FD2	2.4 ± 4.3 a	FD1 = FD2
	FD2	4.0 ± 2.4 a	FD2 = T	4.1 ± 3.1 ab	FD2 = T	3.7 ± 2.7 a	FD2 ≠ T	3.6 ± 3.2 a	FD2 ≠ T
No. of food pellets captured/min	FD1	0.41 ± 0.62 a	FD1 = T A	0.21 ± 0.28 a	FD1 ≠ T A	0.39 ± 0.51 a	FD1 = T A	0.58 ± 0.51 ab	FD1 = T A
	T	0.27 ± 0.40 a	FD1 ≠ FD2	0.45 ± 0.69 b	FD1 ≠ FD2	0.53 ± 0.65 a	FD1 ≠ FD2	0.33 ± 0.34 b	FD1 = FD2
	FD2	1.21 ± 1.12 b	FD2 ≠ T	0.58 ± 0.58 b	FD2 = T	0.91 ± 0.82 b	FD2 ≠ T	0.70 ± 0.78 a	FD2 ≠ T
Mean distance moved to capture food	FD1	36 ± 37 a	FD1 = T N	9 ± 11 a	FD1 = T A	22 ± 19 a	FD1 = T A	34 ± 39 a	FD1 = T N
	T	35 ± 26 a	FD1 = FD2	13 ± 18 a	FD1 ≠ FD2	30 ± 22 ab	FD1 ≠ FD2	14 ± 13 a	FD1 = FD2
	FD2	42 ± 33 a	FD2 = T	26 ± 29 b	FD2 ≠ T	33 ± 24 b	FD2 = T	31 ± 36 a	FD2 = T
Chases of conspecifics/min	FD1	0.08 ± 0.30 ab	FD1 = T A	0.00 ± 0.04 a	FD1 = T A	0.04 ± 0.16 a	FD1 = T A	0.03 ± 0.13 a	FD1 = T A
	T	0.00 ± 0.00 b	FD1 = FD2	0.07 ± 0.19 ab	FD1 ≠ FD2	0.20 ± 0.61 a	FD1 ≠ FD2	0.02 ± 0.09 a	FD1 ≠ FD2
	FD2	0.24 ± 0.64 a	FD2 ≠ T	0.09 ± 0.23 b	FD2 = T	0.48 ± 0.74 b	FD2 ≠ T	0.18 ± 0.50 b	FD2 ≠ T
Chases of heterospecifics/min	FD1	—		—		—		—	
	T	—		—		0.17 ± 0.85		0.0 ± 0.0	
	FD2	—		—		—		—	
Chases of focal individual by a conspecific/min	FD1	0.06 ± 0.19 a	FD1 = T A	0.06 ± 0.25 a	FD1 = T N	0.06 ± 0.24 a	FD1 = T A	0.01 ± 0.06 a	FD1 = T A
	T	0.05 ± 0.29 a	FD1 ≠ FD2	0.05 ± 0.26 a	FD1 = FD2	0.11 ± 0.39 ab	FD1 ≠ FD2	0.05 ± 0.17 ab	FD1 ≠ FD2
	FD2	0.20 ± 0.41 b	FD2 ≠ T	0.05 ± 0.21 a	FD2 = T	0.12 ± 0.27 b	FD2 = T	0.16 ± 0.52 b	FD2 = T
Chases of focal individual by a heterospecific/min	FD1	—		—		—		—	
	T	—		—		0.06 ± 0.19		0.0 ± 0.0	
	FD2	—		—		—		—	
% Time spent by trout with a conspecific within 15 cm	FD1	38 ± 46 a	FD1 ≠ T I	21 ± 38 ab	FD1 = T A	11 ± 24 a	FD1 = T A	29 ± 41 a	FD1 ≠ T A
	T	0 ± 0 b	FD1 = FD2	4 ± 11 a	FD1 = FD2	7 ± 19 a	FD1 ≠ FD2	5 ± 14 b	FD1 = FD2
	FD2	32 ± 40 a	FD2 ≠ T	9 ± 17 b	FD2 ≠ T	21 ± 30 b	FD2 ≠ T	9 ± 15 ab	FD2 = T
% time spent with a heterospecific within 15 cm	FD1	—		—		—		—	
	T	—		—		18 ± 37		0.02 ± 0.10	
	FD2	—		—		—		—	

Conversely, Reeves et al. (1987) observed significant interactions between redside shiner (*Richardsonius balteatus*) and juvenile anadromous rainbow trout. This interaction, however, was strongly influenced by the differential temperature optima of the species, which resulted in partial macrohabitat segregation. Reeves et al. (1987) also found that rainbow trout were behaviorally dominant over redside shiners.

Nonetheless, some fisheries managers believe that interspecific competition with nonsalmonid fishes is a limiting factor for trout in streams (Holey et al. 1979; Moyle 1977). Our data suggest that although trout and dace occupied statistically indistinguishable microhabitats in Coweeta Creek (Grossman and Freeman 1987), there is little evidence that dace exclude trout from their preferred microhabitat. Consequently, it is unlikely that management efforts which target dace as a competitor will result in an increase in trout abundance and biomass. In the southern Appalachian region, it appears that floods and droughts, coupled with strong intraspecific competition, play a much stronger role in regulating trout populations (Durniak and England 1986; Freeman et al. 1988).

At present there is substantial disagreement over the importance of interspecific competition to coexistence and resource utilization in stream fishes. Interspecific competition clearly affects resource use in some species pairs (Baltz et al. 1982; Allan 1986), yet the pattern in many assemblages is one of great overlap with little evidence of interference (Baltz and Moyle 1984; Grossman and Freeman 1987; Grossman et al. 1987a, 1987b; Ross et al. 1987). Our results demonstrate that although there is extensive overlap in microhabitat use between rainbow trout and rosyside dace, there is little evidence that dace affect microhabitat use in trout. This lack of competition may be due to the dynamic nature of many stream habitats (Horwitz 1978; Grossman et al. 1982; Gorman 1988). Survival in such habitats may depend more on the ability to utilize a wide variety of resources than on the exclusion of potential competitors from discrete resource types.

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Calibration of a Gypsum Source for Freshwater Flow Measurements[1]

Ellen L. Petticrew

Department of Fisheries and Oceans, Coastal Oceanography, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2, Canada

and Jacob Kalff

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1, Canada

Petticrew, E. L., and J. Kalff. 1991. Calibration of a gypsum source for freshwater flow measurements. Can. J. Fish. Aquat. Sci. 48: 1244–1249.

A method for estimating flow rate in freshwater was refined by calibrating the rate of dissolution of inexpensive and easily made gypsum cylinders at flows of 0–10 $cm \cdot s^{-1}$ and at temperatures between 3 and 25°C. Gypsum flux was linearly related to flow ($r^2 > 0.94$); thus, weight loss combined with the surface area of the gypsum source and exposure period allows an estimate of flux. Temperature-corrected estimates of flow, obtained from calibration equations and observed dissolution of gypsum cylinders, were virtually identical to flow measured with an electromagnetic current meter in five of eight comparisons conducted in a lake and in three rivers; the three other comparisons were within 0.95 $cm \cdot s^{-1}$. The calibrations are suitable for inexpensive and quantitative estimates of flow in freshwater within the range of temperature and flow conditions typically encountered. We suggest that the method can be used for comparing flow regimes in virtually any marine or freshwater environment as long as salinity and temperature among water bodies are comparable.

Nous avons a amélioré une méthode d'estimation de l'ecoulement en eau douce en étalonnant la vitesse de dissolution de cylindres de gypse, peu coûteux et faciles à fabriquer, à des vitesses de 0 à 10 $cm \cdot s^{-1}$ et des températures allant de 3 à 25°C. Nous avons constaté une relation linéaire entre le flux de gypse et l'écoulement ($r^2 > 0{,}94$); ainsi, en combinant la baisse du poids avec la surface de la source de gypse et la durée d'exposition, on peut estimer le flux. Les valeurs estimatives de l'écoulement, corrigées en considération de la température, que nous avons déterminées au moyen d'équations d'étalonnage et de la dissolution observée étaient pratiquement identiques à l'écoulement mesuré au courantomètre électromagnétique dans cinq des huit comparaisons menées dans un lac et dans trois rivières. Ces étalonnages se prêtent bien aux estimations quantitatives peu coûteuses de l'écoulement en eau douce dans les limites de température et d'écoulement habituelles. Nous proposons cette méthode pour comparer des régimes d'écoulement dans pratiquement n'importe lequel milieu marin ou dulçaquicole, pourvu que la salinité et la température des eaux des milieux étudiés soient comparables.

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Several authors have effectively used hydrated calcium sulfates (such as gypsum, alabaster, plaster of Paris, etc.) as quantitative and qualitative turbulence sensors in both marine and freshwater systems (e.g. Muus 1968; Santschi et al. 1983; Newsome and Aalto 1987). A strong linear relationship between dissolution rate and water flow reported by James and Lupton (1978) indicates that if temperature and salinity were taken into account, gypsum weight loss could be used as an inexpensive but quantitatively reliable measure of average current speeds or as a quantitative index to classify energy environments among water bodies. Qualitative uses of sulfur hydrates to evaluate flow (e.g. Doty 1971; Crabtree and Trudgill 1984; Newsome and Aalto 1987) simply correlated weight loss of the rock source (molds of approximately uniform size and shape) with water flow. However, as the dissolution rate of the material is also a function of surface area and temperature, the weight loss estimates should be corrected or standardized for both of these variables.

In a study to test water flow variation and sediment retention in lacustrine weed beds, we required an inexpensive measure of the relative flows in a multitude of locations in the littoral zone of Lake Memphremagog, Quebec. In this paper we report the results of flume and water bath calibrations of gypsum cylinders at flows up to 10 $cm \cdot s^{-1}$ and over a range of typical lake temperatures. We also provide in situ comparisons of flow rates measured with an electromagnetic current meter and gypsum dissolution rates in currents ranging from 1.5 to 39.7 $cm \cdot s^{-1}$.

Materials and Methods

Theory

Gypsum ($CaSO_4 \cdot 2H_2O$) dissolution is well defined by a first-order kinetic equation of the form (Lui and Nancollas 1971)

$$(1) \quad \frac{dm}{dt} = KA\,(cs - c)$$

where m (kilograms) is the mass of calcium sulfate dissolved at time t (seconds), K (metres per second) is the first-order rate

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constant, A (square metres) is the area of calcium sulfate exposed to the aqueous phase, cs (kilograms per cubic metre) is the saturation concentration of gypsum, and c (kilograms per cubic metre) is the concentration of gypsum in solution at time t.

Theoretically, K, the first-order rate constant, is defined by $D/\S$ where D is the diffusion coefficient (square metres per second) and § is the thickness (metres) of the boundary layer. When the equation is rearranged to represent a flux (F) (kilograms per square metre per second) of gypsum, the equation becomes

$$(2) \quad F = \frac{D}{\S}(cs - c).$$

Several authors (Lui and Nancollas 1971; James and Lupton 1978) indicated that gypsum dissolution is probably governed by a Nernst diffusion mechanism such that the rate-limiting step for dissolution is the transport through the diffusive sublayer rather than the release of ions from the crystal structure. Increases of current speeds are expected to decrease the boundary layer, or alternatively increase the concentration gradient by reducing c: both have the effect of increasing the gypsum flux. Temperature affects the dissolution of gypsum by altering the viscosity of the surrounding water which affects both D and §. Lui and Nancollas (1971) showed the response to temperature variation follows an Arrhenius dependence in that the log of the first-order rate constant, K, decreases linearly with increases in the inverse of absolute temperature, T (degrees Kelvin). The general form of the Arrhenius equation is

$$(3) \quad K = Ae^{-C/T}$$

with A and C being constants representing the intercept and slope, respectively. The rate constant has been shown to be insensitive to variations in pH above 3.9 (Crabtree and Trudgill 1984).

Cylinder Preparation

Various batches (~1 kg each) of commercially available powdered calcined gypsum ($2(CaSO_4 \cdot H_2O)$) were obtained from Westroc of Canada (Montreal). The source rock was a massive evaporite and contained ~15% impurities that may have included clays, silica sand, anhydrite, and limestone. Enough gypsum powder to make two cylinders (50 g) was mixed with distilled water (42 mL) and poured into cylindrical plastic molds (1.9 cm diameter, 9.0 cm height) stoppered at both ends. Stoppers were removed when the mixture had set (~2 h) and the molds were then soaked in hot water (~10 min at 70°C) until a smooth cylinder could be ejected. The cylinders were dried at room temperature and sealed at both ends with epoxy which served to protect the ends of the cylinders from dissolution. Cylinders prepared for use in flows exceeding 7 $cm \cdot s^{-1}$ had a 20-cm piece of threaded stainless steel rod molded 4 cm into one end of the gypsum. This allowed for stable positioning in fast flows during both field and flume calibrations. The cylinders were then oven-dried at 40°C for 48 h and cooled in a desiccator until they were weighed (±0.0005 g) and measured for length and diameter (±0.01 mm). Following their exposure to various flows in the flume and in the field, the cylinders were removed from the water column and were again drip-dried, oven-dried, cooled, and weighed as outlined above. Gypsum weight loss was determined by difference.

It is important to note that the powder to water ratio and the drying temperature are both critical to the successful preparation of cylinders. Excess water results in a cylinder of low mechanical strength. Drying temperatures above 40°C will probably result in a hydrate to anhydrite conversion in the crystalline structure as bound water is removed.

Flow Calibration

We employed two flumes for the calibration of gypsum dissolution. The first was a large flume which was effective for controlling flow over a long distance, thereby allowing many replicates and/or a variety of exposure periods for a single flow. A second smaller flume was designed for use within a 180-L water bath in order that the temperature effects on flow could be ascertained. The first set of dissolution experiments took place in a 9.2-m Siemonsen flow-through (nonrecirculating) tilting flume. The flume channel was 31 cm wide and 45 cm deep. Domestic water was used throughout and water temperature was stable between 3 and 4°C during the period of experiment. The flume was maintained in a level position and water depth and flow were controlled by adjusting the water head in front of the flume entrance and by adjusting the height of the sharp-crested weir at the exit. A series of baffles homogenized the turbulence created by the flow of water from the head tank.

Midchannel, middepth flow was measured with a small middepth drogue transitting a 1-m length of the flume 4 m downstream of the head tank. Flow rates used for the calibration ranged from 0 to 6.3 $cm \cdot s^{-1}$ and were maintained for up to 36.5 h. Gypsum cylinders could be hung at seven positions (between 2 and 8 m downstream of the head tank) along the length of the flume. Three replicate cyclinders were suspended cross-channel (10, 16, and 22 cm from the wall) and 15 cm above the bottom at each site. As many as 27 cylinders were used per flow calibration. Visual examination of the turbulent flow using a dye tracer indicated that cylinder spacing did not result in flow interference among cylinders. The gypsum cylinders were rotated every 12 h (when required) during calibrations to expose the initially downstream side to the upstream side. This was done simply to facilitate an even dissolution and a simpler calculation of postcalibration surface area. As controlled temperature increases could not be performed with this flume, a smaller Plexiglas channel was built for use in a water bath.

Temperature Calibration

A 180-L water bath (0.98 × 0.56 × 0.33 m) was used to maintain the water temperature for the evaluation of temperature and flow effects on gypsum flux. A flume measuring 0.65 × 0.30 × 0.24 m was positioned 10 cm above the floor and centred to the width of the water bath. The back wall of the flume was clamped to an end wall of the water bath which provided a mixing space of 30 cm at the front end of the channel. Water was drawn down the channel toward the back wall by a motorized propeller which extended through a hole in the flume bottom. Baffles 10 cm in front of the propeller and at the mouth of the flume assisted in homogenizing flow. This structure resulted in approximately 45 cm of channel length which was suitable for positioning gypsum cylinders. An evaluation of flow interference between gypsum cylinders indicated that a maximum of four cylinders could be calibrated during each run. Due to the scale of the flume and the spatial distribution of cylinders, the flow was determined separately for each position as slight differences were expected. Flow measurements

TABLE 1. Summary statistics for selected flow, temperature, exposure, cylinder weight, and diameter loss. (A) Flow calibration in the large flume with 3°C water. One standard deviation is provided in parentheses below each average estimate. (B) Temperature calibration in the small flume at zero flow.

(A)	Flow ($cm \cdot s^{-1}$)	Exposure time (h)	Number of samples	Weight loss (g)	Diameter loss (cm)
	2.5	12	6	0.904	0.050
				(0.218)	(0.011)
	2.5	36	9	2.505	0.152
				(0.288)	(0.008)
	3.7	12.5	3	1.157	0.067
				(0.362)	(0.026)
	3.7	36.5	3	3.337	0.198
				(0.562)	(0.031)
	6.3	12	9	1.786	0.096
				(0.298)	(0.017)
	6.3	36	9	4.688	0.291
				(1.268)	(0.078)

(B)	Temperature (°C)	Exposure time (h)	Number of samples	Weight loss (g)	Diameter loss (cm)
	5	24	6	0.697	0.039
				(0.255)	(0.011)
	10	24	4	1.039	0.053
				(0.311)	(0.023)
	20	24	4	1.413	0.068
				(0.801)	(0.045)
	20	48	4	2.665	0.141
				(0.615)	(0.029)
	20	72	4	4.131	0.208
				(0.052)	(0.002)

were obtained using both a Novar streamflow probe and a mid-depth drogue.

To avoid problems of saturating the water with gypsum, the bath was drained between runs, thereby ensuring that concentrations were kept below 15% saturation. Exposure periods of between 5 and 30.5 h were assigned to each run. The exposure period was selected to allow significant cylinder weight loss for the given combination of flow and temperature. Dissolution experiments were run at six temperature settings (3, 5, 10, 15, 20, and 25°C) to provide a range typically found in temperate waters. At each temperature, a zero flux measurement was determined. At 3, 5, 10, 15, and 25°C, a low (~2 $cm \cdot s^{-1}$), medium (~5 $cm \cdot s^{-1}$), and high (>7.5 $cm \cdot s^{-1}$) flow was generated to evaluate the combined effect of flow and temperature on gypsum flux.

In situ Comparisons

In situ water flow rates as measured by the gypsum cylinders were compared with flow rates measured with an Interocean S4 electromagnetic current meter at eight locations within four different water bodies. These included two littoral locations (1.5–2 m depth) in Lake Memphremagog (Vermont–Quebec) and two measurements on each of the Musquodoboit, Avon, and Mersey rivers in Nova Scotia. In the Lake Memphremagog locations, a minimum of four replicate cylinders were moored 22–28 cm above the bottom within 1 m of an Interocean current meter moored at the same depth. At the start of each experiment, each cylinder was anchored to angle iron and suspended with a styrofoam float 20 cm above the cylinder. Each angle

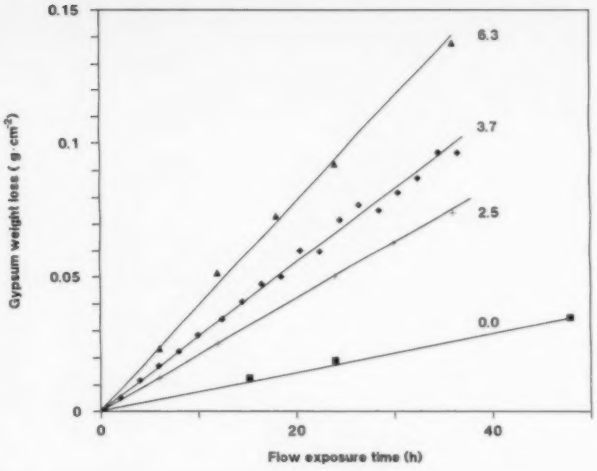

FIG. 1. Relationships between the weight loss of gypsum cylinders and exposure time in 3°C water at four different flow rates (0.0, 2.5, 3.7, and 6.3 $cm \cdot s^{-1}$) in the large flume. Each datum represents the average weight loss per square centimetre of at least three replicate cylinders.

iron with its set of four gypsum replicates was packaged in plastic for ease of transport and delivered and positioned on the lake bottom by a SCUBA diver. Upon retrieval the cylinders were again wrapped in plastic bags to prevent breakage or chipping during delivery to the surface. The water was drained from the bags immediately upon reaching the boat in order to prevent further dissolution.

The current meter recorded current speed, direction, and water temperature. At location 1 the meter averaged the current data over 15-s intervals for 29 h. At location 2 the meter averaged the current data over 5-min intervals for 70 h. Average flow at each location was compared with the flow predicted from the flux of gypsum from the cylinders.

The river flow evaluations required a different gypsum construction, as the plan was to deploy the instruments from a bridge. The gypsum cylinders were prepared with a stainless steel threaded rod moulded into one end. A 70-cm-long crosspiece was attached to the neck of the S4 current meter and a gypsum cylinder was secured to each end. Both cylinders were 20 cm away from the outside edge of the spherical current meter and they were positioned with their midpoint centred at the height of the S4 sensors. On the Musquodoboit River, two gypsum-bearing current meters were hung ~0.8 m above the sand bed river bottom and left for 4.8 and 5.2 h. In each river evaluation, the meters recorded current speed, direction, water temperature, and salinity at 1-min intervals. The river cross-sections were all selected at positions well above the salt water influence, but salinity was measured as a precaution. Two sequential deployments on the Mersey River lasted 2.0 and 1.8 h. The meters were positioned 0.3 m below the water surface several metres in front of a water level regulation weir. The two deployments on the Avon River consisted of 3.4- and 2.0-h periods. The Avon River at this location is regulated by an upstream power plant and during the period of the second deployment the water level dropped, requiring a repositioning of the meter such that it sat on the bed of the river.

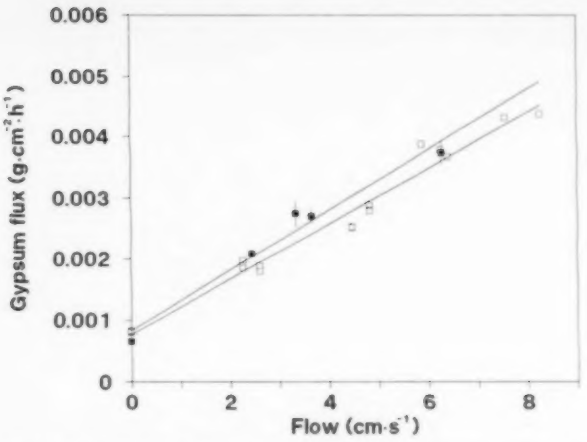

FIG. 2. Relationship between gypsum flux and flow measured at 3°C in two flumes. Solid boxes represent the slope of the weight loss – exposure time relationship (±95% CL) obtained from measurements in the large flume (see equation 4). Open boxes represent measurements from the small flume where $F = 4.55 \times 10^{-4} \times u + 7.56 \times 10^{-4}$, $r^2 = 0.98$.

Results

Flow Calibration

Gypsum weight loss, measured per unit surface area of cylinder per unit time, is subsequently referred to as gypsum flux (F), with units of grams per square centimetre per hour. Weight changes for gypsum cylinders of ~35 cm^2 are of sufficient magnitude to resolve response to flow at temperatures between 3 and 25°C (Table 1). Variations in gypsum flux at all flows (centimetres per second) were associated with neither the downstream nor the lateral position of the cylinders in the large flume (ANOVA, $F = 0.35$, $p = 0.92$, $n = 18$). Weight loss per unit surface area increased linearly with exposure time at each of the flow rates (Fig. 1). Linear regression analysis revealed the relationships to be highly significant at all flows ($r^2 > 0.98$, $p < 0.01$). The gypsum flux determined from the slopes of these lines also increased with increasing flow (Fig. 2). The equation defining gypsum flux (F) as a function of flow (u) in 3°C water is

(4) $F = 8.22 \times 10^{-4} + 4.95 \times 10^{-4} \times u$, $r^2 = 0.97$, $n = 5$.

The 3°C run in the temperature controlled flume (Fig. 2) resulted in a flux–flow equation that was not significantly different from equation 4 (Table 2), even though it was developed in the smaller flume and with a different municipal water supply. The two data sets were pooled to provide a general 3°C flux–flow equation:

(5) $F = 7.94 \times 10^{-4} + 4.57 \times 10^{-4} \times u$, $r^2 = 0.97$, $n = 21$.

Temperature Calibration

The flux–flow relationship is linear at a given temperature. The effect of temperature on both free (zero flow) and forced (flowing water) convection is required for the prediction of flows from gypsum flux data. The form of the flux–flow equation (4 and 5) is

(6) $F = A_T + B_T \times u$

where A_T and B_T, respectively, represent the contribution of free and forced convection to gypsum flux at a given temperature, T. The effect of temperature on both of these can be represented as

(7) $F = (A_0 + q_a \times T) + (B_0 + q_b \times T) \times u$

where A_0 and B_0 are free and forced convection at 0°C and q_a and q_b are the constants representing the effect of each degree of temperature change.

To determine the values of A_0 and q_a the relationship between gypsum flux and temperature at zero flow was determined. Using mean values of flux at six temperatures between 3 and 25°C, the best fit linear relationship was determined:

(8) $A_T = 6.85 \times 10^{-4} + 3.83 \times 10^{-5} \times T$, $r^2 = 0.99$, $n = 6$

where A_0 is 6.85×10^{-4} and q_a is 3.83×10^{-5} (Fig. 3A).

To determine the values of B_0 and q_b the slopes of the flux–flow relationship of the 3, 5, 10, 15, and 25°C runs were determined. They were obtained from the regression equation for each run, as they all exhibited significant linear relationships ($p > 0.01$) (Table 2). The effect of temperature on the flux–flow relationship is evident in the pattern of changing slopes (Fig. 3B). The equation which predicts the flux–flow slope (B_T) or flux per unit flow, as a function of temperature, is

(9) $B_T = 3.92 \times 10^{-4} + 2.10 \times 10^{-5} \times T$, $r^2 = 0.99$, $n = 5$

TABLE 2. Regression results for the flux–flow relationships in both the large and temperature-controlled small flume. The number of samples used in the regression (n) and the standard errors (SE) for both slope and intercept are noted.

Temperature (°C)	Flume	n	r^2	Slope ($\times 10^{-4}$)	SE slope ($\times 10^{-4}$)	Intercept ($\times 10^{-4}$)	SE intercept ($\times 10^{-4}$)
3	Large	5	0.97	4.95	0.49	8.22	2.2
3	Small	16	0.98	4.55	0.18	7.56	1.9
3	Both	21	0.97	4.57	0.18	7.94	2.1
5	Small	12	0.94	4.88	0.37	11.25	4.3
10	Small	11	0.95	6.13	0.46	14.21	4.7
15	Small	8	0.97	7.06	0.54	15.20	4.8
25	Small	13	0.95	9.16	0.61	20.73	7.6

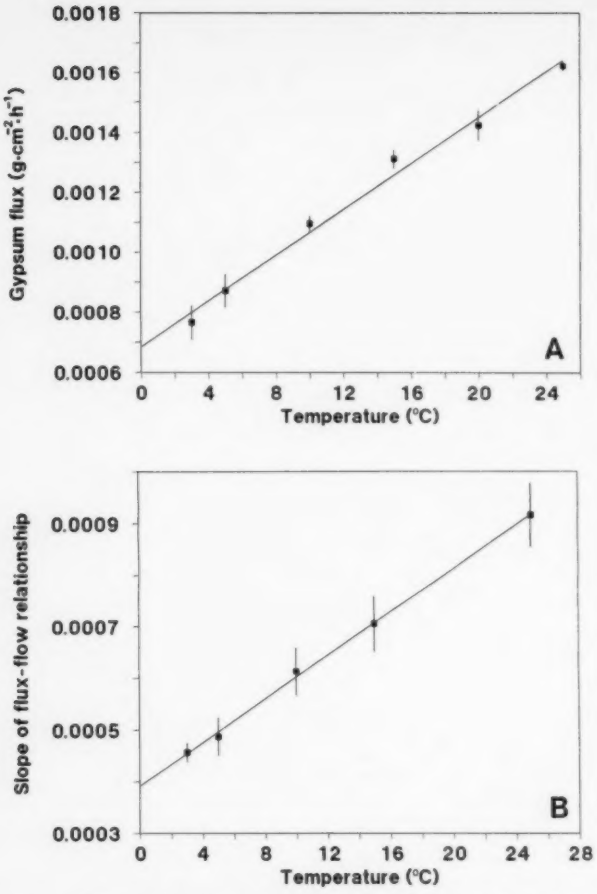

FIG. 3. Effect of temperature on both the intercept and slope of the flux–flow relationship. (A) Gypsum flux and water temperature at zero flow representing the temperature effect on free convection (see equation 8). Each datum is a mean flux ±1 SD. (B) Relationship between the slope of the flux–flow relationships at five temperatures, or the effect of temperature on forced convection (see equation 9). Each slope value is shown with ±1 SE.

where B_0 is 3.92×10^{-4} and q_b is 2.10×10^{-5}. As we would like to predict mean flow from measured gypsum fluxes, equation 6 is rearranged:

$$(10) \quad u = (F - A_T)/B_T.$$

Equations 8 and 9 defining A_T and B_T can be substituted into equation 10 to allow an estimate of mean flow when flux and temperature are known:

$$(11) \quad u = \frac{(F - (6.85 \times 10^{-4} + 3.38 \times 10^{-5} \times T))}{(3.92 \times 10^{-4} + 2.10 \times 10^{-5} \times T)}.$$

In situ Comparisons

The average in situ flow of four different water bodies was estimated using gypsum cylinder flux and temperature in equation 11. At five of the locations, flow estimated with the current meter and with the temperature-corrected gypsum fluxes showed good agreement (Table 3). At locations 1, 2, 5, 6, and 7 the flow, estimated with the current meter for periods between 1.8 and 69.8 h, was within 0.49 cm·s^{-1} of the average flow estimated using gypsum dissolution. At the other three locations the average flow estimated using gypsum dissolution was 0.64 – 0.95 cm·s^{-1} different than that estimated with the current meter.

Discussion

The inexpensive cylinders constructed from commercially available calcined gypsum responded linearly, and with little error, to both flow and temperature variations during the flume calibrations. Field testing of the cylinders indicates that a temperature-corrected flux–flow relationship can be used to predict in situ flows. The eight field comparisons show good correlation between observed and predicted flows, even though four different water bodies were used.

Of interest in the field testing are the large standard deviations noted at the last four locations. Due to irregularities in the local flow patterns the gypsum replicates were measuring different conditions, but as they were equidistant from the S4 sensors, their average flow estimates were similar to the current meter. Flow in the Mersey River could not be considered uniform, as the water level was being regulated by a weir ~2 m downstream of the bridge. The meter was hung from the bridge ~0.3 m below the surface of the water and at a position centred on the weir opening. The flow pattern was not homogeneous, as is evident from the variation in the two replicate gypsum cylinders. While the averages of their flow estimates are similar to the current meter which was positioned between them, it is obvious from both runs on the Mersey River that flow was not uniform over the 70 cm that separated the cylinders. This indicates that variation noted within in situ gypsum replicates can reflect real differences in flow over the scale of the replicate spacing.

The first test on the Avon River had only one usable gypsum cylinder, as the second replicate was chipped upon retrieval. The single useful cylinder result was similar to the flow value of the current meter, which is interesting, as the flow was significantly higher than those tested in the laboratory. The final field test on the Avon River also exhibited a large variance, as the crossbar became slightly tilted with respect to the river bed for part of the run when the meter was lowered through the water column. As the gypsum cylinders were at two different heights in the flow, they exhibit distinctly different fluxes which when averaged are similar to the current meter.

The calibration equation (11) performed well in flows up to ~40 cm·s^{-1} (Avon River) and at temperatures up to ~25°C (Musquodoboit River). The dissolution method also appears effective over a range of periods (2–70 h). Under these field conditions the gypsum flux method resulted in a mean error of 0.47 cm·s^{-1}, with a maximum error of 0.95 cm·s^{-1}. Due to the paucity of field tests between 8 and 35 cm·s^{-1} the validity of flow estimates from gypsum dissolution in high-flow regimes should be further evaluated in freshwater environments.

Caution in comparisons of in situ current measurements is required when water depths may experience contributions of energy from orbital motion imparted from wave action. Orbital motion would be incorporated into the record of mean current by gypsum dissolution whereas it may not be sensed by the electromagnetic current meters, depending on their sampling and averaging scheme. In this set of field comparisons, wind records for each site indicate that orbital motion from wind-

TABLE 3. Summary statistics for eight field experiments comparing flow rates as determined from gypsum dissolution and Interocean S4 current meter results. The number of replicate gypsum cylinders, the period of gypsum exposure to the water column, and the mean temperature are presented. One standard deviation is provided in parentheses below each averaged estimate.

Location	Cylinder No.	Exposure (h)	Temperature (°C)	Current meter flow ($cm \cdot s^{-1}$)	Gypsum flow ($cm \cdot s^{-1}$)
1. Lake Memphremagog	4	28.8	14.1	2.90	2.45
			(0.78)	(0.61)	(0.12)
2. Lake Memphremagog	16	69.8	15.6	1.50	1.01
			(0.21)	(0.37)	(0.20)
3. Musquodoboit River	2	5.18	24.72	3.78	2.83
			(0.15)	(1.22)	(0.05)
4. Musquodoboit River	2	4.80	24.46	3.90	3.26
			(0.15)	(1.75)	(0.19)
5. Mersey River	2	1.95	18.32	5.65	5.66
			(0.04)	(0.70)	(3.05)
6. Mersey River	2	1.84	18.37	5.04	5.26
			(0.09)	(0.94)	(2.67)
7. Avon River	1	3.37	19.70	34.87	34.56
			(0.15)	(4.88)	
8. Avon River	2	2.00	20.01	39.67	38.95
			(0.31)	(5.25)	(11.47)

generated waves would be insignificant at the water depths where gypsum was positioned.

As dissolution occurs, cylinders with epoxy-protected ends decrease in diameter with a concomitant decrease in surface area. The error in flux calculations associated with this changing surface area was calculated for this data set in order to determine if significantly different calibration relationships would result and if the differences in the in situ experimental results could be reduced. When flux values are calculated using the initial measures of surface area the flow estimates are lower than those which incorporate continual surface area changes. The percentage difference in these two calculated fluxes increases as temperature and cumulative flow (flow × time) increase, but in this set of experiments it was always below 8.2%. No significant difference was noted in the slopes or intercepts of the calibration equations when surface area corrections were included in flux calculations. This sensitivity analysis of the effect of changing diameter on surface area indicates that a correction for cylinders of ~35 cm^2 does not appear to be necessary for the range of flows, temperatures, and time periods involved in this series of experiments. In order to ensure that this error is small in high-temperature or high-flow environments the initial surface area or size of the cylinder could be increased.

The calibrations provided in this paper are suitable for estimating flow rates in freshwater averaged over periods as long as 70 h within the range of temperature and flow conditions typically encountered (3–25°C, 0–40 $cm \cdot s^{-1}$). Although the flow calibrations were performed with an upper limit of 10 $cm \cdot s^{-1}$ the relationship appears to hold at higher flows. James and Lupton (1978) found a linear relationship between a different gypsum dissolution method and flow rates as high as 75 $cm \cdot s^{-1}$. If quantitative estimates are to be made in marine systems, a similar calibration procedure incorporating salinity variations must be conducted. Nevertheless, the evidence presented here and in James and Lupton (1978) indicates that gypsum dissolution provides a simple and inexpensive method for relative comparisons of flow regimes in virtually any marine or freshwater environment as long as salinity and temperature among water bodies are comparable.

Acknowledgements

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Abundance and Freshwater Migrations of the Anadromous Parasitic Lamprey, *Lampetra tridentata*, in a Tributary of the Fraser River, British Columbia

Richard J. Beamish

Department of Fisheries and Oceans, Pacific Biological Station, Biological Sciences Branch, Nanaimo, B.C. V9R 5K6, Canada

and Colin D. Levings

Department of Fisheries and Oceans, Biological Sciences Branch, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6, Canada

Beamish, R. J., and C. D. Levings. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary of the Fraser River, British Columbia. Can. J. Fish. Aquat. Sci. 48: 1250–1263.

The migration of young adult *Lampetra tridentata* out of the Nicola River during 1984–85 through 1987–88 was estimated to be approximately 176 000, 19 000, 90 000, and 102 000; at the same time, approximately 749 000, 909 000, 920 000, and 605 000 large ammocoetes, age 4 and 5 yr, left the river. Migration of young adults started in September, with the largest number migrating from mid-March to mid-May. Ammocoetes behaved similarly except that movement continued throughout the year. Most young adults metamorphosed at age 4 or 5 and were age 5 or 6 when they migrated to sea. It was not determined when lamprey from the Nicola River reached salt water; however, *L. tridentata* entered the Strait of Georgia from the Fraser River from March until July. Maturing adults first returned to the Nicola River from the ocean in August when water levels in the river were at their lowest. This is the first estimate of abundance of young adult *L. tridentata* in any river in the Fraser River drainage. The very large number of young adults and older ammocoetes leaving the Nicola River indicate that parasitic lamprey are abundant in the Fraser River and that they are potentially a common predator of commercially important fishes.

Selon nos estimations, environ 176 000, 19 000, 90 000 et 102 000 jeunes *Lampetra tridentata* de stade adulte sont sortis de la rivière Nicola de 1984–1985 à 1987–1988; durant la même période, il est aussi parti environ 749 000, 909 000, 920 000 et 605 000 grands ammocètes de 4 et 5 ans. La migration des jeunes adultes a commencé en septembre; les plus gros contingents ont migré de la mi-mars à la mi-mai. Il en a été de même pour les ammocètes, sauf que leur migration s'est poursuivie toute l'année. La plupart des jeunes adultes se sont métamorphosés à 4 ou 5 ans et avaient 5 ou 6 ans lorsqu'ils ont migré vers l'océan. Nous n'avons pas déterminé quand les lamproies de la rivière Nicola arrivent en eau salée; nous savons néanmoins que *L. tridentata* est entré au détroit de Géorgie, arrivant du Fraser, de mars jusqu'à juillet. Les adultes en maturation ont commencé à migrer de l'océan jusqu'à la Nicola en août, lorsque le niveau de l'eau de la rivière était au plus bas. C'est la première fois que l'on estime l'abondance du jeune *L. tridentata* de stade adulte dans une rivière du bassin du Fraser. Le fait que le nombre de jeunes adultes et d'ammocètes plus âgés quittant la Nicola soit aussi élevé indique que la lamproie parasite est très abondante dans le Fraser et qu'elle pourrait être un prédateur répandu de poissons commercialement importants.

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The Pacific lamprey, *Lampetra tridentata*, is a wide-ranging anadromous parasitic lamprey that occurs in coastal rivers from southern California to the Aleutian Islands in Alaska. In British Columbia, Pacific lamprey occur in most rivers, including the large Fraser River and Skeena River drainages (R. J. Beamish, unpubl. data). Despite the wide distribution in British Columbia, little is known about its abundance or the number and type of prey that are killed during the marine feeding phase. This lack of knowledge probably is related to the difficulty of observing or capturing metamorphosed lampreys.

Pacific lamprey reside as ammocoetes in the fine sediments in shallow backwater areas of streams for approximately 5 yr before they metamorphose into the adult form (Russell 1986; Beamish and Northcote 1989). After migrating to sea between the fall and the spring, Pacific lamprey feed on marine fishes for at least 1 yr (Beamish 1980). Adults return to fresh water in the spring. After migrating into headwater areas, adults spawn and die in the following spring (Pletcher 1963; Beamish 1980; Farlinger and Beamish 1984). It is only during brief periods in the upstream migration and the few weeks of spawning that metamorphosed Pacific lamprey are easily observed.

The relative absence of observations has created the impression that this lamprey is not commonly found and consequently is relatively unimportant as a source of mortality of commercially important fishes. If abundance of either downstream or upstream migrating lamprey were known, it would be possible to begin to estimate the relative importance of lamprey preda-

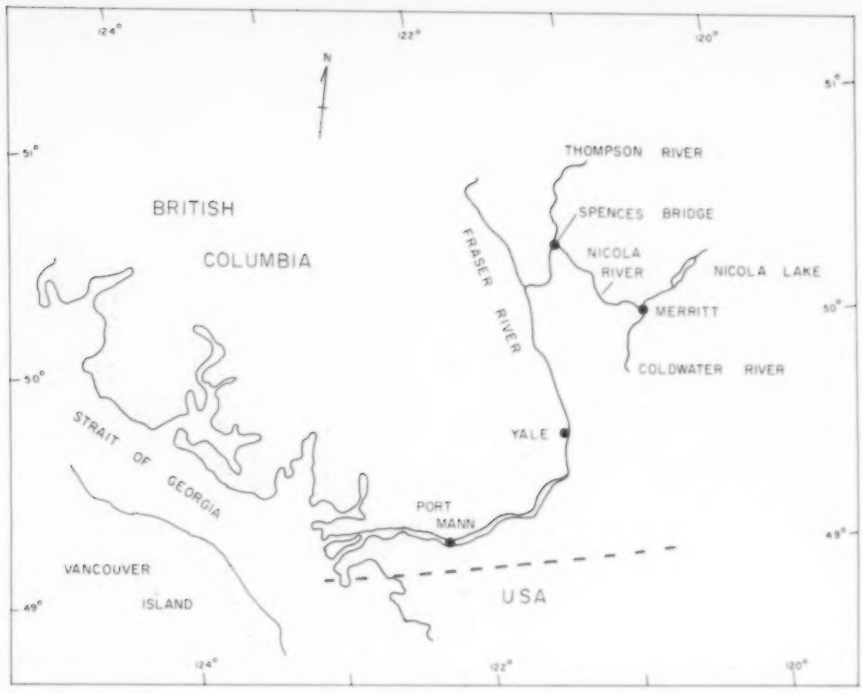

FIG. 1. Location of study and sampling sites.

tion. Unfortunately, it is still difficult to catch lampreys during the upstream migration of adult Pacific lamprey. It is possible to sample the downstream migration. Downstream-migrating lamprey are often captured with salmon. As part of a study of winter movements of juvenile salmon, we extended sampling beyond the normal period of juvenile salmon trapping and obtained information about lamprey movements and abundance.

The information collected in this study was obtained during a study of chinook salmon, *Oncorhynchus tshawytscha*, in the Nicola River (Levings and Lauzier 1989). Sufficient data were obtained to provide the first estimates of abundance of young adult Pacific lamprey from a major west coast river. In addition, we obtained information on the timing of migration in and out of the Nicola River, a tributary of the Thompson River which flows into the Fraser River. The Fraser River has the largest drainage area in British Columbia of approximately 230 400 km^2 and contributes more salmon to the commercial fishery than any other watershed. The Fraser River is 1253 km long with more than 300 rivers and streams in the watershed. Because Pacific lamprey are frequently found in the same rivers as salmon, we suspect that Pacific lamprey occur in a number of these 300 rivers.

Materials and Methods

Migrating lamprey were trapped using floating inclined plane traps (Conlin and Tutty 1979) with a 0.61×0.92 m (2×3 ft) opening. The stainless steel mesh of the inclined plane and live box has a 4-mm mesh size. The traps were suspended from a block and cable system attached to a bridge near the town of Spences Bridge, B.C. The bridge over the Nicola River is 300 m upstream of the confluence of the Nicola and Thompson rivers. Sampling began on March 21, 1985, and continued until freeze-up on November 9, 1985. Sampling resumed on March 10, 1986, and continued until December 1, 1986. Sampling resumed on February 9, 1987, and terminated on May 15, 1987. In 1988, two sites were sampled. Sampling at the mouth of the Nicola River started on March 10 and continued until August 19. Inclined plane trap sampling was also carried out from February 18 until July 20 on the Nicola River at Merritt (Fig. 1). This site is 4 km above the confluence of the Nicola and Coldwater rivers. Except during extreme freshets, two traps were used at Spences Bridge, one within 2 m of the shoreline and another closer to the centre of the river, about 5 m from shore. The block and cable system allowed adjustment of the position of each trap in the river to fish slow or fast water as streamflow conditions changed. During freshet, only the trap closest to the stream bank was used because debris has to be continually removed. Under average flow conditions, the river was approximately 20 m wide. At Merritt the river was usually 8 m wide.

Under normal conditions, traps were checked and catches were enumerated in the morning (06:00–09:00) and evening (18:00–21:00). Surface water temperature and water height were taken in the morning.

At extreme freshet, the trap was fished between 21:00 and 02:00 because of excessive debris. Results of 24-h test fishing during freshet conditions gave the proportion of lamprey caught in a particular time during the 24-h period. The catch of a reduced sample period was divided by the calculated expected proportion for a particular flow regime for a 24-h period, resulting in a standardized 24-h catch.

Total numbers of lamprey leaving the river were estimated using the volume passing through the traps. A Marsh-McBirney electromagnetic current meter (model 201) was used to measure

water velocities at both sides and at the centre of the trap mouth. The water volume was calculated from the depth of the trap mouth floor and the water velocities. Nicola River streamflow was obtained from the Water Survey of Canada gauge No. 08LG006 located 13 km upstream of the trap site. There is negligible addition of water from tributaries between the gauging station and the trap site. The volume passing through the trap was divided by the discharge of the Nicola River to determine the proportion of the Nicola River discharge the traps were sampling.

The 24-h catch was divided by the fraction of discharge sampled by the trap to estimate the total number of migrants. We assumed a homogeneous distribution of lamprey across the river and averaged the catches from the two traps. On days the traps were not fished, a mean was taken of the previous day's estimate and the following day's estimate.

Samples of young adult lamprey and ammocoetes were collected during the periods of maximum migration and preserved in 5% formalin. Specimens were measured and examined for sex in the laboratory. Sex was determined by visual examination and a subsample was checked histologically. Live young adults and ammocoetes were sampled in the laboratory for age determination. Statoliths were removed using the procedure of Volk (1986). Age was determined using the criteria of Volk (1986) and Russell (1986). Ages of lamprey sampled after January 1 were assigned assuming a January 1 birthday. This means that a statolith from a lamprey sampled in April that had a translucent edge and had five opaque zones or annuli was aged as 6 yr. All ages were assigned without knowledge of the size of the lamprey. Ages were determined by two readers, and any statoliths that were not aged identically were reexamined. Final ages were compared with the lengths of the lamprey. At this stage, 15% of the age estimates appeared anomalous and were aged again.

A banding pattern was most obvious when statoliths were examined immediately after they were removed from the lamprey and placed in immersion oil. The position of the statoliths that produced the optimal contrast between translucent zones and opaque zones was found by manipulating the statolith within the beam of transmitted light (see Beamish and Northcote (1989) for photograph of a statolith).

Two samples of young adults were collected (February 2 at Merritt and March 8 at Spences Bridge) from the traps for age determination in 1988. Lamprey from the February sample were weighed and sex was determined in addition to sampling the statoliths and measuring lengths. Samples of ammocoetes and young adults were collected from the mouth of the Nicola River using electroshockers on October 8–11 and 23–24, 1986, and measured without preservation. The combined length–frequency of lamprey collected by electroshocking was aged using a running average of five. Statoliths were removed from a few of the smaller ammocoetes. Ages estimated from these statoliths were not recorded using the January 1 birthday because it was assumed growth was completed, that is, a 1+ ammocoete was recorded as age 2. Adjusting the age estimate in this manner facilitates the comparisons of growth and age at metamorphosis in this study.

In 1986, adults migrating upstream were captured at a site 6 km upstream of the mouth of the Nicola River. A modified fyke net was set on the margin of the river with the wings and opening facing downstream. The wings were made of 0.5-cm wire mesh and the trap was covered with netting of similar mesh size. The width of the wings at the widest point fished approx-

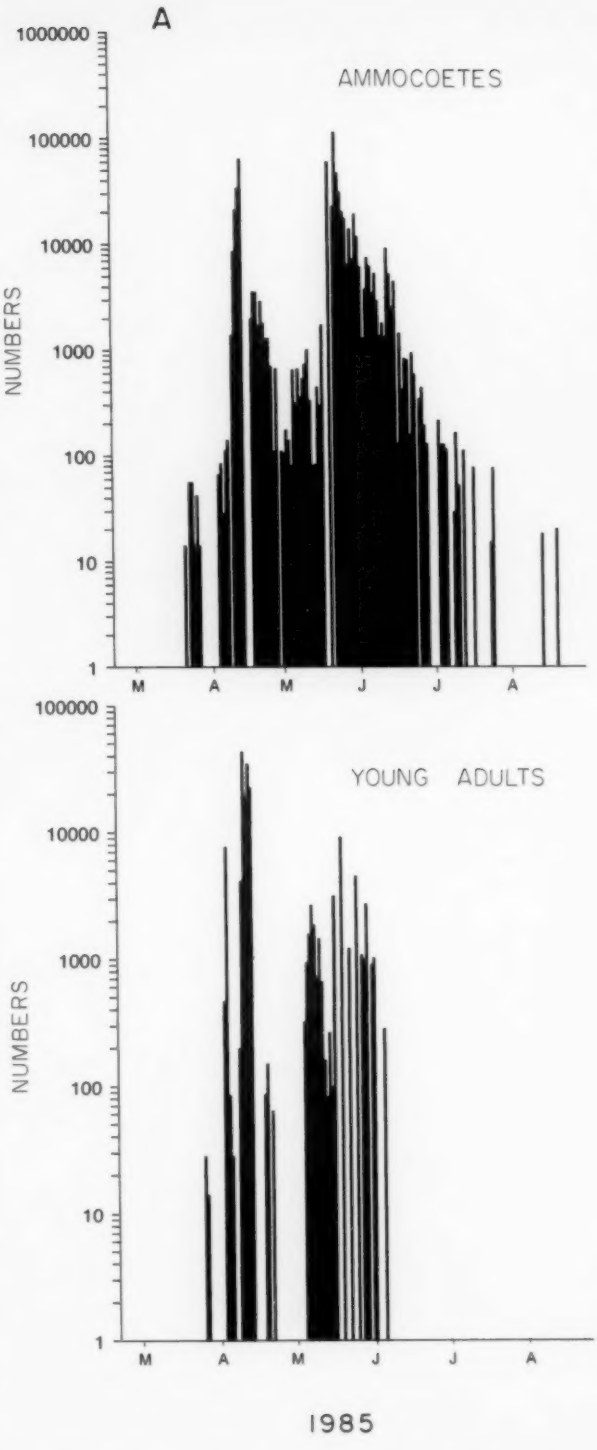

FIG. 2. Calculated daily numbers of young adults and ammocoetes migrating downstream. (A) 1985; (B) September 1, 1985, to August 31, 1986; (C) September 1, 1986, to August 31, 1987; (D) 1988; (E) 1988 at the Merritt site. (*Fig. 2 continued next page*)

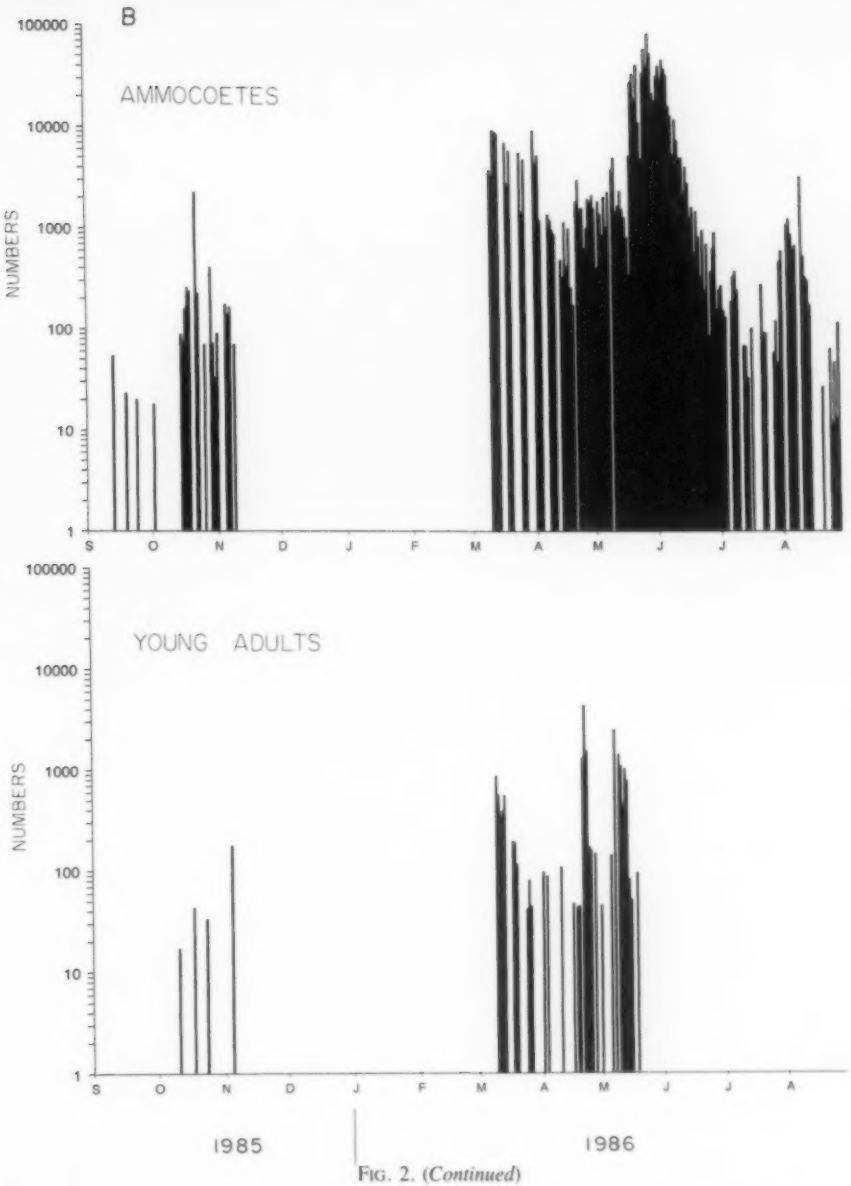

FIG. 2. (*Continued*)

imately 10% of the width of the river. The trap was operated from July 25 until mid-October and was checked daily.

Mature adults migrating up the Fraser River were sampled in 1983 at Yale, 170 km from the mouth of the Fraser River and 118 km downstream from the mouth of the Nicola River. Adult lamprey were jigged as they migrated upstream along the edge of the river in a steep gorge. Jigs were 2-m poles with 8–10 three-pronged fish hooks lashed along the top of the pole.

Lamprey were also collected in 1988, 36 km from the mouth of the Fraser River at Port Mann. A surface trawl with a 0.3-cm codend mesh was fished from February 25 until August 3. The net was fished twice a week according to the procedures described by Whitehouse et al. (1989).

Results

Young Adults

Downstream migration

Lampetra tridentata begin metamorphosis in the summer and complete metamorphosis, including the ability to osmoregulate in salt water, in the fall (Richards and Beamish 1981). Short periods of downstream migration of young adults started in September. In 1985 and 1986 the first downstream migrations occurred on October 11 and September 25, respectively (Fig. 2B, 2C). It is unknown if migration out of the river continued under the ice (November–February 1985–86). In 1986, the ice

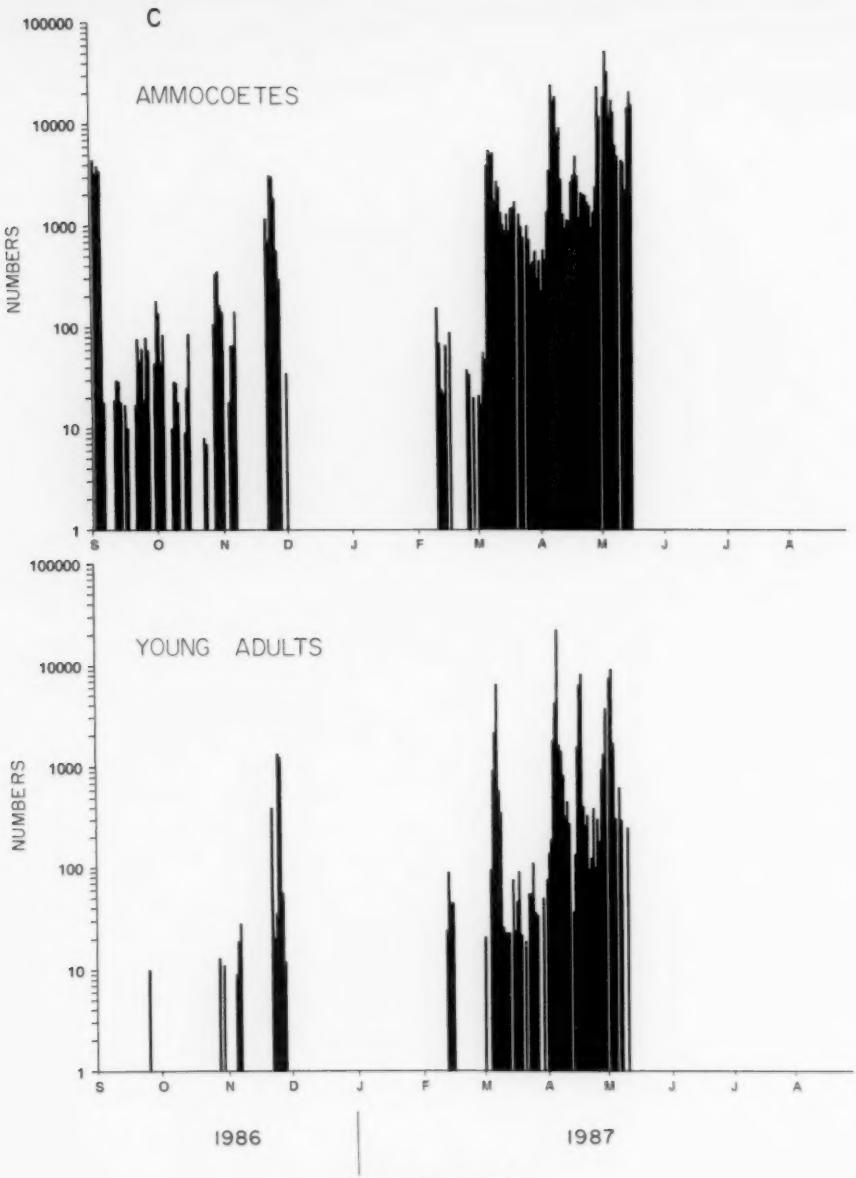

FIG. 2. (*Continued*)

left the river in November after a brief freezing period in early November. Trapping during this ice-free period caught young adults. Traps installed shortly after ice breakup in the late winter immediately captured young adult lamprey. The earliest catches after ice left the river occurred in mid-February in 1987 and 1988 (Fig. 2C, 2E). In 1985 (Fig. 2A), 99% of the all young adults left the river in April and May; 82% left during these two months in 1985–86 (Fig. 2B) and it is possible that a similar pattern of migration occurred during these two months in 1986–87 (Fig. 2C). In 1988, 99.5% of the young adults passed by the trapping site at the mouth of the Nicola River (Fig. 2D) and 92% at the Merritt site (Fig. 2E) in April and May. The downstream migrations from 1985 to 1987 ended abruptly in mid-May or early June. In 1988, metamorphosed lamprey were captured up to July 29 at Merritt, when trapping stopped (Fig. 2E). However, no lamprey were caught in July or August downstream at Spences Bridge.

The correlation between the numbers of downstream migrants, morning temperature, and discharge was examined for the spring–summer migration in 1985 (Fig. 3, 4). The multiple regression identified discharge as the only parameter that showed a significant relationship with the young adults migrating each day ($p < 0.01$). For example, in 1985 the downstream migrants were most numerous on April 3–5, April 10–16, May 5–13, and May 16–22. Abrupt increases in discharge occurred on April 3 and 10 and May 7 and 17 (Fig. 3). None of the parameters showed a significant relationship with ammocoete migration ($p > 0.05$). At Merritt, the large

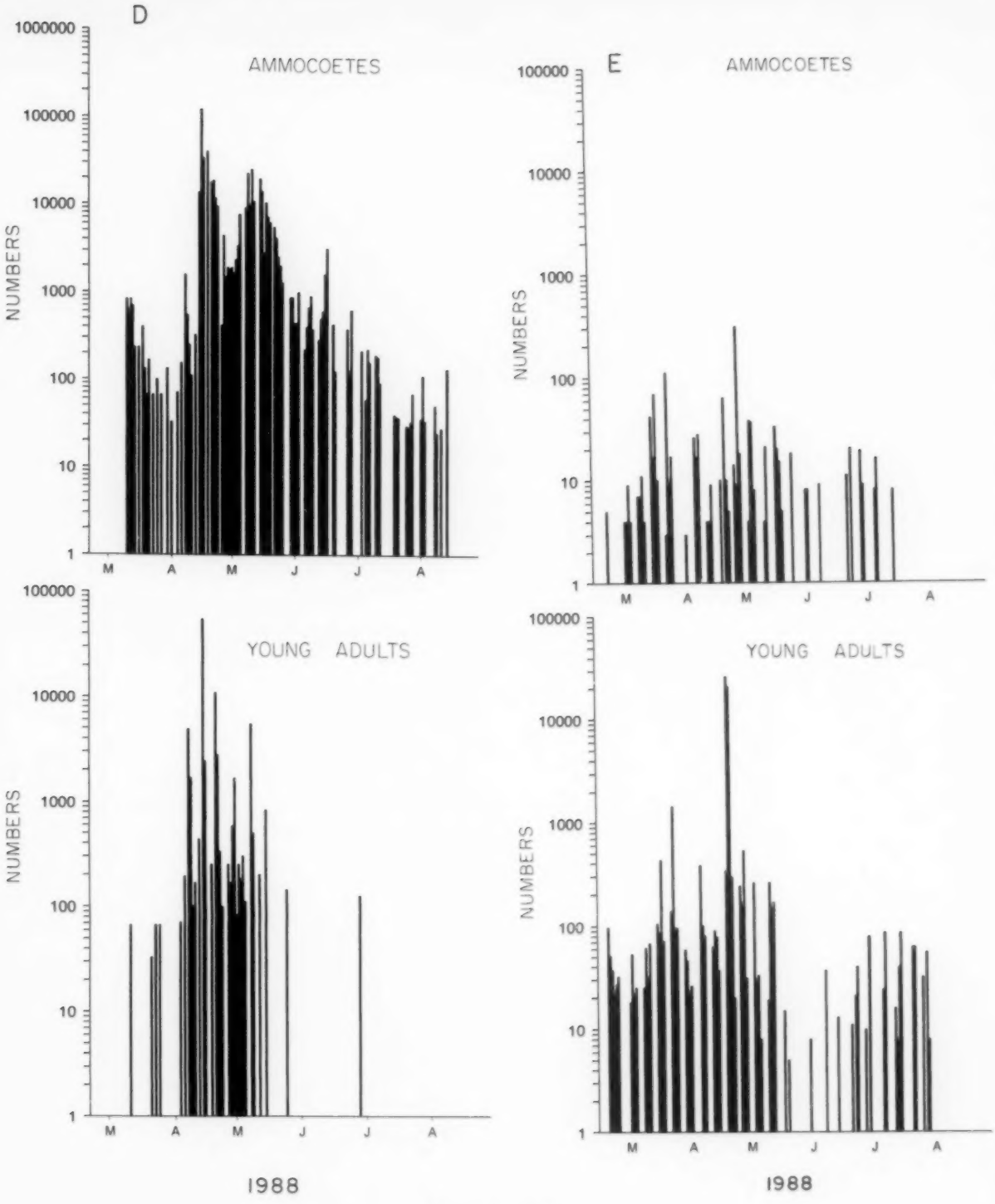

FIG. 2. (*Concluded*)

migrations on April 20 and 21 were not preceded by major increases in discharge. Discharge at this site is controlled by a dam on Nicola Lake.

Lengths

In 1985, the average length of 1331 young adults collected throughout the spring migration was 12.1 cm. In 1986, a sam-

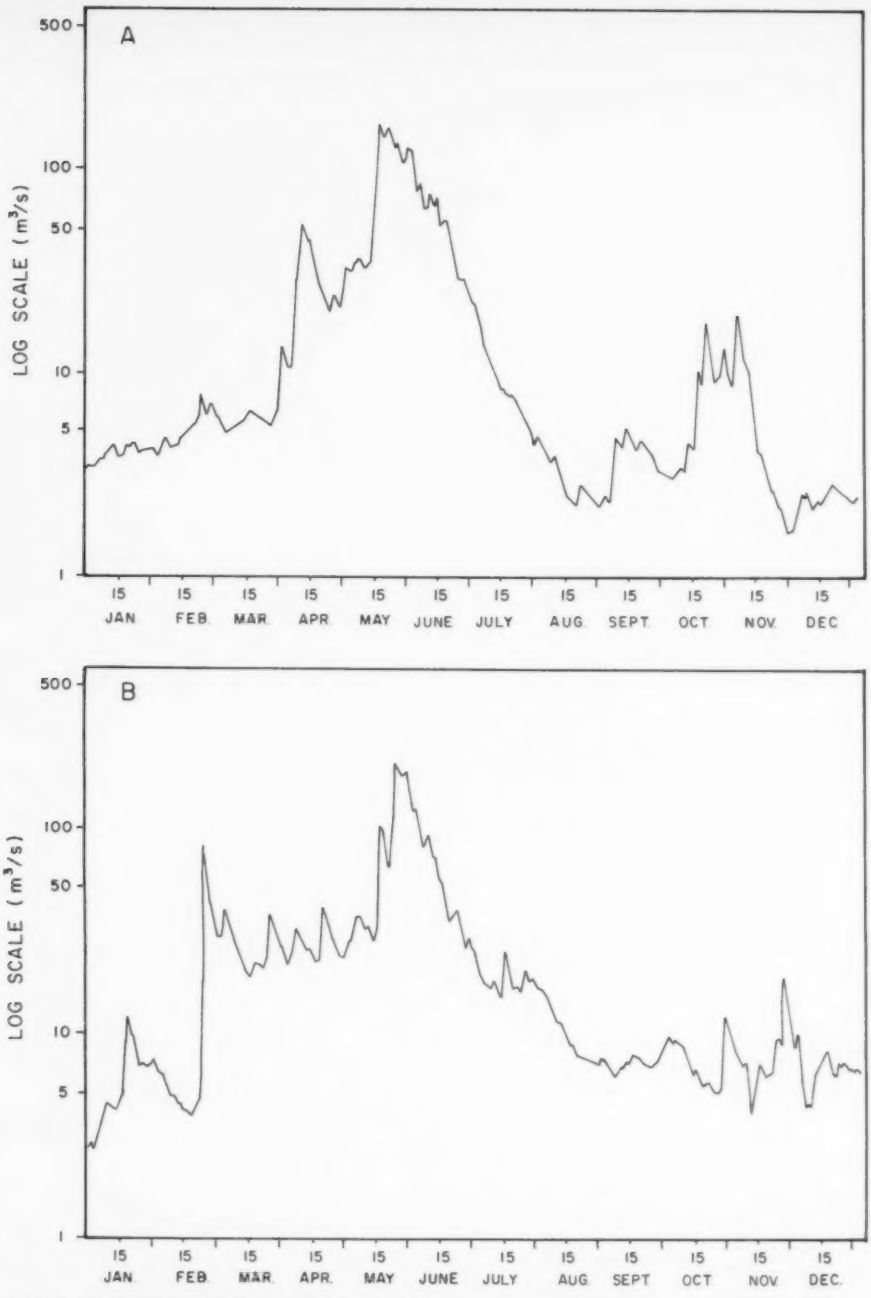

FIG. 3. Nicola River discharge at Spences Bridge as measured by the water survey of Canada. (A) 1985; (B) 1986; (C) 1987; (D) 1988. (*Fig. 3 concluded next page*)

ple of 240 young adults collected from March to mid-May was 10.6 cm. In 1987, 698 lamprey sampled from early April until early May had an average length of 12.3 cm. In 1988 the mean lengths of a sample of 412 young adults from the Merritt trap and 67 from the Nicola River trap were 14.0 and 13.7 cm, respectively. The length–weight relationship for a sample of 651 young adults sampled in April 1987 was $W = 8.5 \times 10^{-6} \times L^{3.093}$.

Abundance

Catches at night accounted for 99% of the total catches. There was considerable variation in the estimated number of young adult lamprey that left the river over the 4-yr study. The migration in 1984–85 was the largest, with an estimated 175 968 young adults leaving the river (Table 1). Because there was no trapping in the fall of 1984, this estimate would underestimate the total number leaving the river. In 1985–86, 1.4% of the

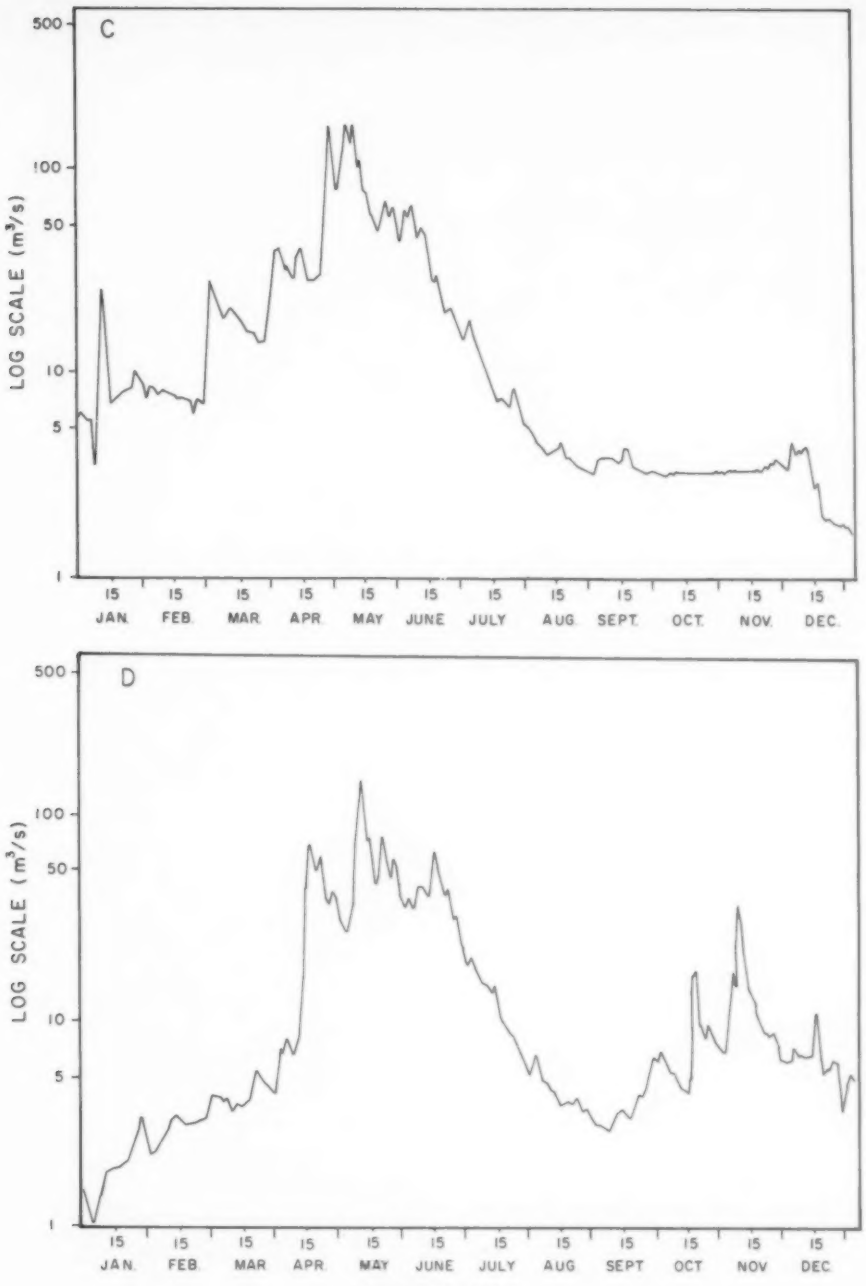

FIG. 3. (*Concluded*)

migration occurred in the fall, and in 1986–87 at least 3.4% occurred in the fall. If the average percentage of these two migrations occurred in 1984–85, there would be 4400 more young adults or a total of 180 367. In 1985–86 the number of young adult migrants was 19 283 or 11% of the previous migration. In 1986–87, 72 199 young adults left the river by the end of April. Because traps were not fished in May and because the catches in May in the previous two years were quite high, we estimated that the 1986–87 estimate was low by about 36%, the average percentage migration for this period in the previous two migrations. An adjusted estimate of approximately 98 191 is approximately one half of the 1984–85 migration and five times larger than the 1985–86 migration.

In 1988, an estimated 99 352 young adults migrated out of the river. This estimate includes the lamprey that were sampled at Merritt and not returned to the river. Because there was no sampling in the fall of 1987, an estimate of the fall migration was made using 2.4% of the total migration, as was done for

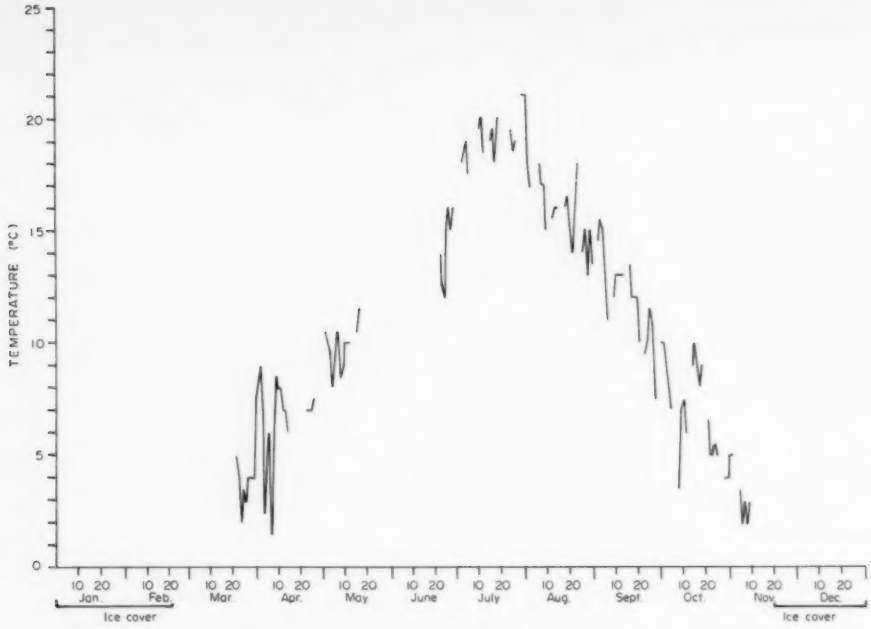

FIG. 4. Temperature at Spences Bridge, 1985.

the 1985 migration. When this estimate is added to the total, there were 101 736 young adults that left the river.

Ammocoetes

The mean length of approximately 3000 ammocoetes measured was 11.7 cm. Approximately 8% were smaller than 100 mm and less than 1% ranged from 75 to 90 mm.

In contrast with young adults, ammocoetes moved out of the river at all sampling times throughout the year (Fig. 2). Large numbers of ammocoetes moved at the same time as most young adults, but the largest numbers moved downstream in May and June. This movement occurred after the main migration of young adults. We added the mean number leaving the river in the fall of 1985–86 and 1986–87 to the 1985–86 and 1987–88 migrations because no observations were made in the fall of 1985 and 1987. We also added the mean number leaving in the spring of 1985 and 1985–86 to the number leaving in 1986–87. The total estimated numbers of ammocoetes were 749 210 for 1984–85, 908 985 for 1985–86, 920 328 for 1986–87, and 605 032 in 1987–88 (Table 1).

In 1988, 1383 ammocoetes were captured in the traps at the mouth of the Nicola River, 318 of which (23%) were captured at night. At the Merritt site, 68% (172) of the total catch of 254 ammocoetes were captured at night. In 1988, at the mouth of the Nicola River, there were approximately six times as many ammocoetes as young adults. At Merritt, there were approximately 43 times as many young adults as ammocoetes.

Lower Fraser River Sampling

Both metamorphosed *L. tridentata* and *Lampetra ayresi* were caught in the trawl sampling, 36 km upstream from the mouth of the Fraser River. A total of 97 lamprey were captured from March 25 until August 8, 1988: 28 metamorphosed *L. tridentata*, 56 metamorphosed *L. ayresi*, eight *L. ayresi* ammocoetes, and seven that were not saved for identification (Table 2). Most lamprey (67) were captured between April 17 and 20 in both day and night sets.

Age Determination

A total of 93 young adults and 33 ammocoetes were used for age determination, six of which could not be aged. The first annulus was located in an area of the statolith where an abrupt change in the shape occurred. This abrupt change frequently formed a distinct notch in the profile of the statolith. If there was doubt about the location of the first annulus, it was assumed to be located in this notch. In general, the opaque zones were clear for ages up to 3 and 4. The opaque zones were closer together, and contrast between opaque and translucent zones was reduced for older ages.

Metamorphosed lamprey ranged in age from 4 to 8 (Table 3). The mean lengths of young adult lamprey aged 5, 6, 7 were not significantly different (t-test, $p > 0.05$). Most lamprey began metamorphosis at age 4+ or 5+ and were age 5 or 6 when sampled in the year following metamorphosis (Table 3). The average length of the few young adults that were estimated to be age 7 and 8 was smaller than those lamprey that metamorphosed at a younger age (Fig. 5). One lamprey metamorphosed at age 3+. Sex was only determined from the March 1988 sample. Of 66 young adults sampled March 1988, 30 were female and 36 were male. There was no significant difference in the average lengths of male and female lamprey of similar age (t-test, $p > 0.05$). However, there were more age 7 males than females (Table 4).

The oldest ammocoetes were age 6. The average size of age 5 ammocoetes was significantly smaller (t-test, $p < 0.05$) than the average size of age 5 metamorphosed individuals.

The length–frequency analysis indicated that more ammocoetes would be in the older age groups than indicated by the

TABLE 1. Numbers of young adult lamprey and ammocoetes estimated to move out of the Nicola River.

	1984–85				1985–86				1986–87				1987–88							
													Young adults				Ammocoetes			
	Young adults	%	Ammocoetes	%	Young adults	%	Ammocoetes	%	Young adults	%	Ammocoetes	%	Merritt	%	Nicola	%	Merritt	%	Nicola	%
September	—		—		0		97	—	10	0.01	15 641	6.9	—		—		—		—	
October	—		—		93	0.5	3 898	0.4	23	0.03	1 379	0.6	—		—		—		—	
November	—		—		174	0.9	639	0.1	2 468	3.4	9 835	4.3	—		—		—		—	
December	—		—		—		—		—		—		—		—		—		—	
January	—		—		—		—		—		—		—		—		—		—	
February	—		—		—		—		—	0.3	514	—	417	0.7	—		5	0.4	—	
March	42	0.02	196	—	3 202	16.6	60 347	6.6	11 327	15.7	43 943	19.3	3 368	0.6	333	0.3	343	26.3	5 084	0.9
April	133 215	75.7	149 397	20.4	6 737	34.9	42 415	4.7	58 171	80.6	156 285	68.7	50 593	89.4	89 146	89.7	610	46.8	358 290	60.8
May	40 518	23.0	503 247	68.6	9 077	47.1	468 223	51.5	—		—		1 410	2.5	9 748	9.8	220	16.9	211 620	35.9
June	2 193	1.3	79 493	10.8	0		319 939	35.2	—		—		232	0.4	125	0.1	94	7.2	15 384	2.6
July	0		1 095	0	0		3 055	0.3	—		—		544	1.0	0		32	2.6	1 493	2.5
August	0		38	—	0		10 372	1.1	—		—		—		0				417	0.1
Total	175 968		733 466		19 283		908 985		72 199		227 597		56 563		99 352		1 304		589 288	
Estimated annual abundance	180 367		749 210		19 283		908 985		98 191		920 328				101 736				605 032	

statolith method (Tables 3, 5; Fig. 6). Specifically, the length–frequency method indicated that the largest percentage of ammocoetes in the sample should be age 7 (Table 5; Fig. 6). Because ages produced using the length–frequency analysis were not similar to ages estimated from the statolith method, we had to select one age determination method and reject the other. We based our analysis on the statolith method because length–frequency analysis is a less accurate method (Beamish and McFarlane 1983).

Adults

Adult lamprey migrating upstream were first caught at Yale (Fig. 1) on July 25, 1983. Records of lamprey collected by a commercial sturgeon fishermen (lamprey are used for bait for sturgeon fishing) for 3 yr prior to our sampling indicated that the upstream migration first occurred at this site at the end of July or the first week in August. On all nights, we caught lamprey from 22:30 to 03:00. On July 25, 50 lamprey were caught, and on July 30, over 100 lamprey were captured. Commercial fishing continued after our sampling until mid-August when catches became quite small. The total lengths of a sample of 48 adults ranged from 27.3 to 45.3 cm, with an average length of 33.7 cm and weight of 72.7 g. There were equal numbers of males and females.

The first adult lamprey in the Nicola River were captured on August 14, 1986. Over the period August 14–28, seven females and one male were captured with an average length of 30.0 cm and weight of 55.9 g. No more lamprey were captured until October 1–7 when two females and two males were captured with an average length of 27.7 cm and weight of 50.7 g. The trap was removed in mid-October.

Discussion

Migration of Young Adults

There are only a few studies of the downstream migration of young adults and ammocoetes that have monitored movements throughout the year. One of the earliest and most extensive studies (Applegate 1950) reported that recently metamorphosed landlocked sea lamprey, *Petromyzon marinus*, began moving out of rivers late in October or early in November. Movement continued throughout the winter, with most movement occurring in late March and early April. Migratory activity was closely associated with increases in discharge and not with temperature changes. This association between increases in discharge and the initiation of downstream movement of juvenile adults has been reported for a number of other species: *L. ayresi* (Beamish and Youson 1987), *Geotria australis* (Potter 1980), *Mordacia mordax* (Potter 1970), and anadromous *P. marinus* (Beamish and Potter 1975). In this study, discharge initiated downstream movement of young adults even though movement at the Merritt site started without a major fluctuation in flow rate. We think that the migration at Merritt indicates that even small increases in flow rate are sufficient to initiate downstream migration.

The pattern of a small migration in the fall followed by a spring migration of metamorphosed *L. tridentata* is similar to patterns observed from other species (Applegate 1950; Bird and Potter 1979). The migration in the Nicola River, therefore, started as early as late September in one year and continued to late May or early June in the next year except in 1988 when a small migration occurred late in July. Any bimodality in the

TABLE 2. Catches of lamprey at the mouth of the Fraser River.

	L. tridentata	*L. ayresi*	Not identified	*L. ayresi* ammocoetes
February 25	1	—	—	—
April 6	1	—	—	—
April 19–28	10	6	2	—
May 5–6	0	6	1	—
May 10–15	3	9	—	—
May 16–19	5	23	1	2
May 25 – June 20	6	9	—	4
June 24 – July 4	2	3	—	2
August 8	—	—	1	—
Total	28	56	5	8

TABLE 3. Age composition of Nicola River *L. tridentata* (combined sexes).

	Age (yr)	*n*	Mean length (mm)	SD	Range in length (mm)
Ammocoetes	2[a]	4	60	4.5	56–66
	3[a]	8	80	6.7	80–100
	4	6	115	11.8	98–132
	5	13	115	7.6	100–127
	6	2	119	4.2	116–122
Adults	4	1	127	—	—
	5	37	138	10.2	117–157
	6	35	140	11.5	118–175
	7	10	132	13.2	113–156
	8	3	121	10.6	113–133

[a]From electroshocking sample.

pattern of migration appears to be a result of stream conditions and the timing of observations rather than two distinct and separate migration periods.

In this study and others, downstream migration occurred mostly at night (Long 1968; Hardisty and Potter 1971; Potter and Huggins 1973; Potter 1980). For example, at the Merritt trap in 1988, 2060 young adults were captured from April 15 to 17. All but four were captured at night. By the time young adults were close to the mouth of the Fraser River, they migrated both at night and during the day. Movement throughout the 24-h period at the mouth of the Fraser River was also reported for *L. ayresi* (Beamish and Youson 1987).

The migratory behaviour near the mouth of the Fraser River differed from that observed in the Nicola River. Young adult *L. tridentata* were not caught until late March and the daily catches were more uniform over the period of migration that occurred from March 25 until July 4. Thus, the characteristics of the migration depend on the sampling location. At the beginning of the migration, in the headwaters of the various rivers, young adults begin the downstream migration at night when discharge increases abruptly. Most leave the substrate over a very short period. As the migration proceeds towards salt water, more groups amalgamate, resulting in a more uniform migration that continues day and night. In this study, the young adults that left the stream first were longer than those that migrated later. We also observed that young adults were smaller in some years that others.

Migration of Ammocoetes

Ammocoetes that move out of the Nicola River do not migrate into salt water because they are unable to osmoregulate

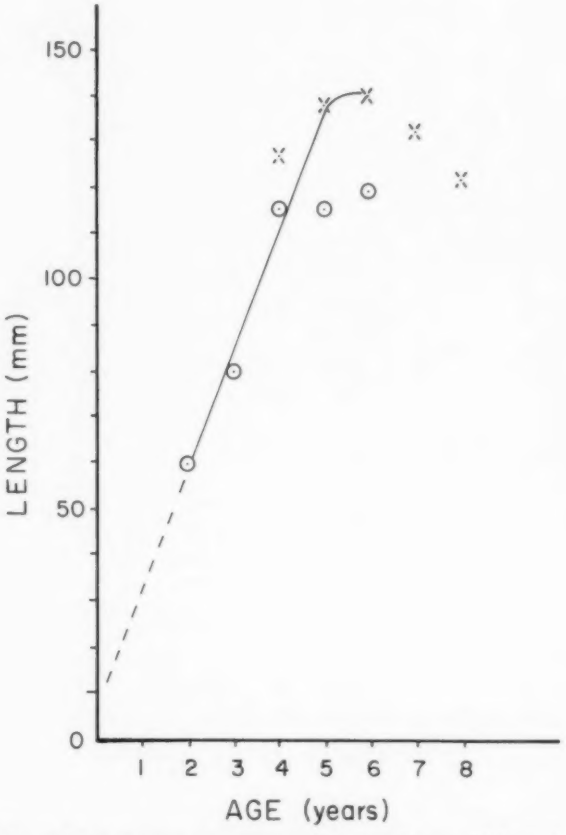

FIG. 5. Mean size at age of ammocoetes (○) and young adults (×).

TABLE 4. Mean length of male and female young adult lamprey.

Age (yr)	Mean male length (mm)	*n*	SD	Mean female length (mm)	*n*	SD
4	127	1	—	—	—	—
5	142	16	7.7	140	13	7.1
6	141	13	7.1	143	16	11.3
7	135	6	8.5	158	1	—

TABLE 5. Comparison of ages of ammocoetes using statoliths and length–frequency analysis.

Age (yr)	Statolith mean length (mm)	Length–frequency mean length (mm)
1	—	33
2	60	64
3	80	74
4	115	84
5	115	94
6	119	105
7	—	115
n	33	685

in salt water (Hardisty 1956; Morris 1972). It is believed that this downstream movement disperses larval lamprey throughout the watershed and is common among lamprey (Potter 1980). We did not observe a seasonal movement, as observed in other studies (Manion and Smith 1978; Potter 1980; Gritsenko 1968); however, most downstream movement occurred in the spring when the discharge was the highest, consistent with other studies (Manion and McLain 1971; Manion and Smith 1978). Smaller numbers of ammocoetes moves out of the river in the summer even though flows were reduced, water levels were low, and temperatures were high. The 10°C "barrier" reported by Manion and Smith (1978) for the Big Garlic River study was not observed in our study. Movement of ammocoetes occurred primarily at night, as observed in other studies (Gritsenko 1968; Manion and Smith 1978; Potter and Huggins 1973).

It is interesting that migration of young adults was almost always associated with an increase in abundance of larger ammocoetes moving downstream except in 1988 at the Merritt site. In the upper Nicola above Merritt, discharge was very constant in April 1988, as a consequence of the controlled flow from Nicola Lake (Fig. 1). The relative absence of any ammocoete movement may be related to discharge or may indicate low ammocoete abundance in this area. If there are few ammocoetes, the large number of young adults could only be produced by an upstream migration of ammocoetes prior to metamorphosis or an upstream migration of young adults prior to their downstream migration. We think that this is unlikely because of the strong tendency for ammocoetes to move downstream. We believe that the controlled flow in the area of the Merritt traps did not provide the stimulation for ammocoetes to move downstream. Therefore, larger increases in discharge may be required to stimulate movement of ammocoetes than required to stimulate the migration of young adults.

Age

Ages assigned using statoliths have not been validated. An indication that accurate ages may be produced by this method was found in a study of a population of *L. tridentata* that was prevented from going to sea (Beamish and Northcote 1989). The method was also valid for younger age groups of some populations of some other species of lamprey (Medland and Beamish 1987). However, the differences in mean size and age estimates between the statolith and length–frequency age determination methods indicate that the ages produced by the statolith method must be validated before it is considered to be accurate.

The age composition of the population, estimated using statoliths, was similar to the population of *L. tridentata* from the Ash River (Beamish and Northcote 1989). The mean lengths of similarly aged lamprey were larger for lamprey from the Nicola River than the Ash River, but the ages at metamorphosis and the number of age groups of young adults were similar.

In this study, there was an indication of a relationship between slower growth and age at metamorphosis. The average size of some lamprey that metamorphosed at an older age was

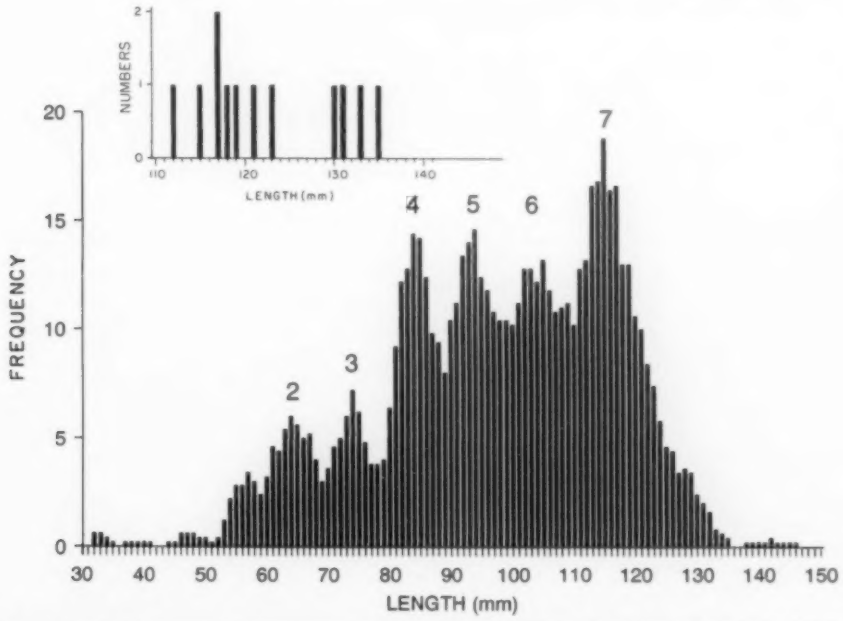

FIG. 6. Length–frequency of ammocoetes using a running average of five. Age estimates are shown above the modes. Lengths of metamorphosed lamprey (n = 12) are inserted in the upper left corner.

smaller than those that metamorphosed earlier, suggesting that slower growing ammocoetes may metamorphose at older ages.

The existence of four age groups in the metamorphosing population indicates more age groups of young adult Pacific lamprey than the two age classes reported for young adult *Lampetra fluviatilis* (Potter and Huggins 1973). The presence of at least two major age groups from the four age groups indicates that the interannual variation in the abundance of young adults, discussed earlier, may be related to environmental conditions. In the absence of a fishery and with the inevitable influence of the environment on survival, it is unlikely that a population can maintain cycles of abundance when young adults are from more than one age group. Unless cycles are produced by an association with cyclic abundance of predators, it is probable that the variation in the numbers of young adults leaving the Nicola River is related to conditions within the river.

Spawning Migration

Adult *L. tridentata* first returned to the Nicola River in August. This was unexpected because water levels were very low and it might be expected that adults would not enter the river until discharges were higher. For example, in the Chemainus River, adults entered the river when discharge was close to its annual maximum (Richards 1980). If the lamprey sampled at Yale in August form any part of the migration into the Nicola River, lamprey move upstream from Yale to the Nicola River, approximately 112 km, in about 2 wk or about 8 km/d. This would suggest that the lamprey entered the Fraser River, 170 km downstream of Yale, towards the end of June. This is about the time of maximum discharge of the Fraser River and would indicate that lamprey may in fact return during maximum discharges, as observed in the Chemainus River, but reach the Nicola River at low discharge.

Length of Life after Metamorphosis

Using the growth rates determined in the laboratory (R. J. Beamish and C.-E. Neville, unpubl. data), it would take about 12 mo for young adult lamprey to reach the size of the adults sampled at Yale. Assuming that metamorphosis is complete by about the end of September, the average young adult would spend 8 mo in fresh water, enter the sea in April–May, spend about 12–14 mo in salt water, returning to fresh water in June or July. It would then spend about 4 mo returning to the Nicola River and 7–9 mo more in fresh water before spawning and dying. The length of life after metamorphosis, therefore, is about 32 mo or approximately 2.5 yr.

Abundance

The number of young adults leaving the Nicola River varied considerably among the four years. The 1985 migration of approximately 176 000 was the largest and about 9 times greater than that of 1985–86. The 1986–87 and 1987–88 migrations were of intermediate size. We are not aware of any studies that have estimated the abundance of anadromous lamprey migrating out of larger rivers. Thus, it is unknown if the large variation in abundance is common. To estimate the potential amount of predation on marine fishes by young adult lamprey produced in the Nicola River, we considered the average migration to be 100 000 young adults.

The abundance of young adult lamprey produced in the Nicola River is the sum of the number of young adults leaving the river and those produced by the ammocoetes that left the river in previous years. Most ammocoetes leaving the river were age 4 and 5, about 1 yr from metamorphosis. There are no estimates of mortality that can be used to estimate how many of these ammocoetes survive and metamorphose. However, for most fishes, the period of highest mortality occurs immediately after hatching, suggesting that mortality rates may not be high at age 4 and 5. We used a very high mortality rate of 0.5 to estimate the total production of young adult lamprey so that it would not appear that we are attempting to inflate the estimate of young adults produced. If about one half of the average migration of about 800 000 ammocoetes leaving the river survive and metamorphose, then these young adults can be added to the average migration of about 100 000 young adults. The total annual production of young adults would be approximately 500 000.

If the abundance of *L. tridentata* relative to *L. ayresi* is representative of the actual proportion in all years, then it is also possible to estimate the total numbers of *L. tridentata* using abundance estimates for *L. ayresi* (Beamish and Youson 1987). In 1979, it was estimated that 6 500 000 young adult *L. ayresi* left the Fraser River. In this study, approximately one *L. tridentata* was captured for every two *L. ayresi*, indicating that total *L. tridentata* abundance in the Fraser River drainage may be about 3 000 000 young adults. Obviously this estimate is little better than a guess; however, until proper abundance estimates are made, it is useful to have some appreciation of the numbers of *L. tridentata* that leave the Fraser River and to show that the Nicola River is a major producer of *L. tridentata* in the Fraser River drainage.

Impact on Commercial Fishes

There are no laboratory studies that identify the feeding preference of Pacific lamprey. In laboratory studies that compared the preference between salmon and herring, it was observed that more herring were eaten than salmon (R. J. Beamish, unpubl. data). Scarring information (Gilhousen 1989; Beamish 1980) indicated that salmon were attacked by Pacific lamprey, but the relationship between scarring and mortality was unknown.

The relative importance of Pacific lamprey as predators can be assessed by comparing their abundance with the abundance of landlocked sea lamprey (*P. marinus*) in the Great Lakes because the impact of this landlocked sea lamprey is known and because the Pacific lamprey and the anadromous form of the sea lamprey may be similar in their predatory behaviour. At the peak of the destruction of the commercial fishery in the 1950s, approximately 750 000 spawning sea lamprey were in three Great Lakes (Lake Huron, Lake Michigan, and Lake Superior; Walters et al. 1980). There are no recent estimates of abundance for all of these lakes, but an estimate of spawning lamprey in 1987 in all of Lake Superior was 47 662 (Dustin et al. 1988). Our estimate of 3 000 000 young adult Pacific lamprey leaving the Fraser River each year or 500 000 young adults produced by the Nicola River does not account for marine phase mortalities. If we use an 80% mortality rate used to estimate the number of feeding landlocked sea lamprey that survive to spawn (Walters et al. 1980), the Nicola River would produce 100 000 spawning lamprey and the Fraser River 600 000 spawning lamprey. If the average number of spawning adults produced by the Nicola River is compared with the Great Lakes, this single river produces approximately twice the number of

spawning lamprey currently in Lake Superior. The estimate of 600 000 spawning lamprey produced by the Fraser River is 80% of the estimate of the maximum numbers of spawning lamprey in all three Great Lakes, during the peak abundance in the 1950s. The high abundance relative to landlocked sea lamprey abundance in the Great Lakes indicates that the Pacific lamprey should be recognized as an important predator of marine fishes.

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Classification of Fish Assemblages based on Echo Integration Surveys

Laura J. Richards, Robert Kieser, Timothy J. Mulligan, and John R. Candy

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada

Richards, L. J., R. Kieser, T. J. Mulligan, and J. R. Candy. 1991. Classification of fish assemblages based on echo integration surveys. Can. J. Fish. Aquat. Sci. 48: 1264–1272.

Echo integration data typically collected during fish biomass surveys have additional utility in species recognition. Our analysis is based on a rockfish (family Scorpaenidae) hydroacoustic survey off the northwest coast of Vancouver Island, British Columbia, Canada. Survey data from 105 echogram sections were divided into two categories on the basis of ocean bottom habitat. One category corresponded to an area of bedrock outcrops and the other to the continental slope adjacent to the shelf break. Hydroacoustic characteristics of the bottom-oriented fish assemblages associated with these habitats could be described by time of day, mean volume density, dispersion, and mean off-bottom distance of the biomass. Nearest-neighbour analysis of these features classified assemblages into habitat categories with up to 97% success. Between-habitat differences in the assemblages were also evident from concurrent experimental fishing. Different rockfish species dominated the assemblages in the two areas. Our results suggest general methods for distinguishing among species groups in hydroacoustic survey data.

Les données d'échointégration généralement recueillies dans les relevés de populations de poissons peuvent aussi servir à l'identification des espèces. Notre analyse est fondée sur le relevé hydroacoustique d'une sébaste (famille des scorpénidés) au large de la côte nord-ouest de l'île de Vancouver, en Colombie-Britannique (Canada). Nous avons groupé les données de 105 échogrammes en deux catégories en fonction de la nature du fond océanique. La première catégorie correspond à une zone d'affleurements du socle rocheux et la seconde, à la zone du talus continental adjacente à la rupture de la plate-forme. Les caractéristiques hydroacoustiques des assemblages de poissons associés au fond qu'on trouve dans ces habitats peuvent se décrire par l'heure, la densité volumique moyenne, la dispersion et la distance moyenne des poissons par rapport au fond. Avec l'analyse de fréquence des bases contiguës, il a été possible de classer les assemblages par catégorie d'habitat avec un taux de réussite atteignant 97 %. Les résultats de la pêche expérimentale réalisée en même temps ont aussi fait ressortir des différences entre les assemblages d'habitats différents. Les sébastes dominant les assemblages étaient différentes dans les deux zones étudiées. À la lumière de nos résultats, nous pouvons proposer des méthodes générales permettant de distinguer les espèces d'après les données d'hydroacoustique.

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Hydroacoustic techniques are traditionally applied to fish abundance estimation, in terms of either numbers (echo counting) or biomass (echo integration) (Clay and Medwin 1977; Ehrenberg and Lytle 1972; Mitson 1983). Echo integration is the more appropriate of the two techniques for schooling fish. The procedure involves echo sounding along a transect and measuring the returned echo intensity. A calibrated hydroacoustic system then provides an estimate of the fish biomass for specified depth and distance intervals along the transect (Burczynski 1982; Forbes and Nakken 1972). A resolution of 10-m depth by 250-m distance, for example, is appropriate for most fish population surveys.

Traditionally, hydroacoustic biomass surveys have depended on concurrent fishing to identify the fish species observed. Although fishermen often distinguish schools of different fish species on the basis of echograms, quantifying such visual impressions has proved difficult. Most attempts at fish species recognition have relied on special acoustic equipment (Holliday 1980; Simmonds and Armstrong 1987) and detailed analysis of the echo signal (Dickie et al. 1983; Nash et al. 1987; Rose and Leggett 1988). Recently, however, Nero and Magnuson (1989) described echo integration data that they obtained from 1-m depth by 25-m distance intervals. They found features of these data to be useful in discriminating fish and invertebrate assemblages from several different water masses. Consequently, such high-resolution data may enable classification of fish schools to at least taxonomic group.

The purpose of this paper is to demonstrate that typical echo integration data, such as those collected during fish surveys, can also be applied in species recognition. Indeed, such data can be sufficient to discriminate among fish schools of different species or species groups. We begin by using echograms to identify sections of hydroacoustic data from the two major habitats in the study area. We then define variables from these data that describe the bottom-oriented fish schools in the two habitats. In the language of pattern recognition, the echogram sections are termed objects, and features are the variables defined from the hydroacoustic measurements on each object. In the final step, we use nearest-neighbour analysis (NNA) on the features to classify the objects into habitat categories. Our approach provides a major improvement over subjective interpretation of echograms and, after preliminary processing, can be implemented with standard statistical software.

All classification algorithms require the category of each object to be known outside of the data. Here, for example, the habitat categories are known independently of the hydroacoustic measurements on the fish schools. Similarity among objects is assumed to decrease as the distance among objects increases in feature space. NNA (Duda and Hart 1973; Hand 1981) determines the category of an unclassified object from a simple rule; it belongs to the same category as the majority of its L nearest neighbors, where L is a value selected by the user. With two categories, an odd value of L is normally chosen to avoid ambiguity in the decision rule. One of the advantages of NNA is that it represents a leave-one-out cross-validation procedure (Hand 1981, chap. 8). Thus, cross-validation is automatic and the object being classified is not used in constructing the decision rule. This differs from discriminant analysis, where two sets of objects are required, one for constructing the decision rule and a second for performing the classification.

Our work was motivated by a fisheries management issue. Trawl fishermen questioned whether the fish schools they observe on their sounders over rocky, untrawlable areas comprise the same rockfish species (family Scorpaenidae) as those available to them on the trawl grounds. The economic consequences of this information are considerable, due to restrictive quotas on the trawl catch of many rockfish species (Leaman 1990). By NNA, we find differences between the two habitat categories in the features determined from the hydroacoustic signal. The classification results are supported by experimental fishing, as different rockfish species dominate the fish assemblage in each habitat (Matthews et al. 1989).

Methods

Data Collection

Echo integration data were collected in Brooks Bay (50°14′N latitude, 128°15′W longitude), on the west coast of Vancouver Island, Canada, during a cruise on the CSS *John P. Tully* in March 1989. Complete cruise details are given in Matthews et al. (1989). The data analyzed here were selected from transects conducted between March 16 and 18, 1989. On these dates, a sequence of four transects, each 6 km in length, was surveyed continuously over a 39-h period, providing 15 repetitions of each transect. Transects were located 1 km apart and oriented perpendicular to the 200-m depth contour. Bottom depths ranged from approximately 50 m to greater than 275 m along each transect.

Each 6-km transect covered a region of pinnacles and a region surrounding the continental shelf break (Fig. 1). Data corresponding to the pinnacle and slope regions were chosen as objects for the NNA, representing the rocky area of interest to fishermen and the trawl grounds, respectively. The four transects provided four replicates of each object. Since transects were surveyed 15 times, we obtained $4 \times 15 = 60$ measurements of each object for a total of 120 sets of measurements. Features determined from these sets of measurements were used by NNA to classify the objects into habitat categories.

The hydroacoustic equipment included a Simrad ceramic transducer, a Biosonics echo sounder with a stable time varied gain ($20 \log R + 2\alpha R$), and a Biosonics digital echo integrator that was serially linked to a personal computer. A digital audio video recorder provided a recording of the echo signal. The transducer was mounted in a torpedo-shaped body and towed behind the vessel at a speed of 8 knots (14.8 km/h) and at a depth of approximately 25 m. Hence, all depths given below were obtained by adding 25 m to the recorded range. The transducer emitted a 38-kHz pulse of 0.6-ms duration, with a pulse repetition rate of 50 pulses/min. Returned signals were echo integrated in 10-m depth and 1-min time (approximately 250-m distance) intervals over the 110-m depth range from 65 to 175 m. A dry paper chart recorder superimposed these integration boundaries on the echograms. To prevent integration of the bottom echo, signals were integrated to a point 2.5 m off the bottom.

Definition of the pinnacle and slope objects from sections of the echogram is illustrated in Fig. 1. Although some variation in elapsed time does occur, 5 min was the approximate time required to survey most pinnacle regions. Thus, pinnacle objects are defined by 5-min echogram sections centered over the bedrock outcrops. To ensure valid comparisons, 5-min sections are also identified for slope objects, defined by the 5-min section that includes the 155-m depth contour at the shallow end of the shelf break. Bottom depth is below the integration range for three of the slope time intervals in Fig. 1. Since the echo integrator did not record depths below 275 m, bottom depth is not known for the deepest intervals. This does not pose a problem for the analysis, as we demonstrate below.

Preliminary Data Processing

Echo integration averages the ping to ping echo intensities obtained for each object k ($k = 1,\ldots, s$) over specified depth intervals i ($i = 1,\ldots, n$) and time intervals j ($j = 1,\ldots, m$). The echo integrator output I_{ijk}, the volume density v_{ijk}, the echo integration constant K, and the backscattering cross-section σ of the insonified fish are related by the equation

$$(1) \quad I_{ijk} = K\sigma v_{ijk}$$

(Forbes and Nakken 1972; Clay and Medwin 1977). Hence, the volume density can be obtained from

$$(2) \quad v_{ijk} = I_{ijk}/K\sigma.$$

The constant K is determined during calibration and is described for our equipment in Kieser et al. (1987). The value of σ is somewhat arbitrary. We assumed a target strength of -32 dB/kg (Dunn 1979; Forbes et al. 1980; Kieser 1983).

For the pinnacle and slope objects, we have defined $n = 11$ depth intervals and $m = 5$ time intervals. Thus, for each object k, the volume densities v_{ijk} can be represented as an 11×5 matrix. The mean depth R_{jk} for each column j is also output from the echo integrator. In addition, the echo integrator provides an ancillary matrix of values f_{ijk}, representing the fraction of the 10-m depth/time interval ijk located above bottom. For most elements ijk in the ancillary matrix, $f_{ijk} = 1$. At deeper depths, as the bottom begins to encroach into the volume represented by v_{ijk}, values f_{ijk} are in the range (0,1). For matrix elements located completely below bottom, $f_{ijk} = 0$. Three of the four features that we define below are statistics of the volume density matrix and the ancillary output from the echo integrator.

The ability to distinguish fish biomass from background noise and ocean bottom is a fundamental problem with acoustic data. For this reason, preliminary processing is usually required before features can be computed. In particular, depth and time intervals must be identified for which volume densities v_{ijk} measure fish targets of interest. There are six reasons for elim-

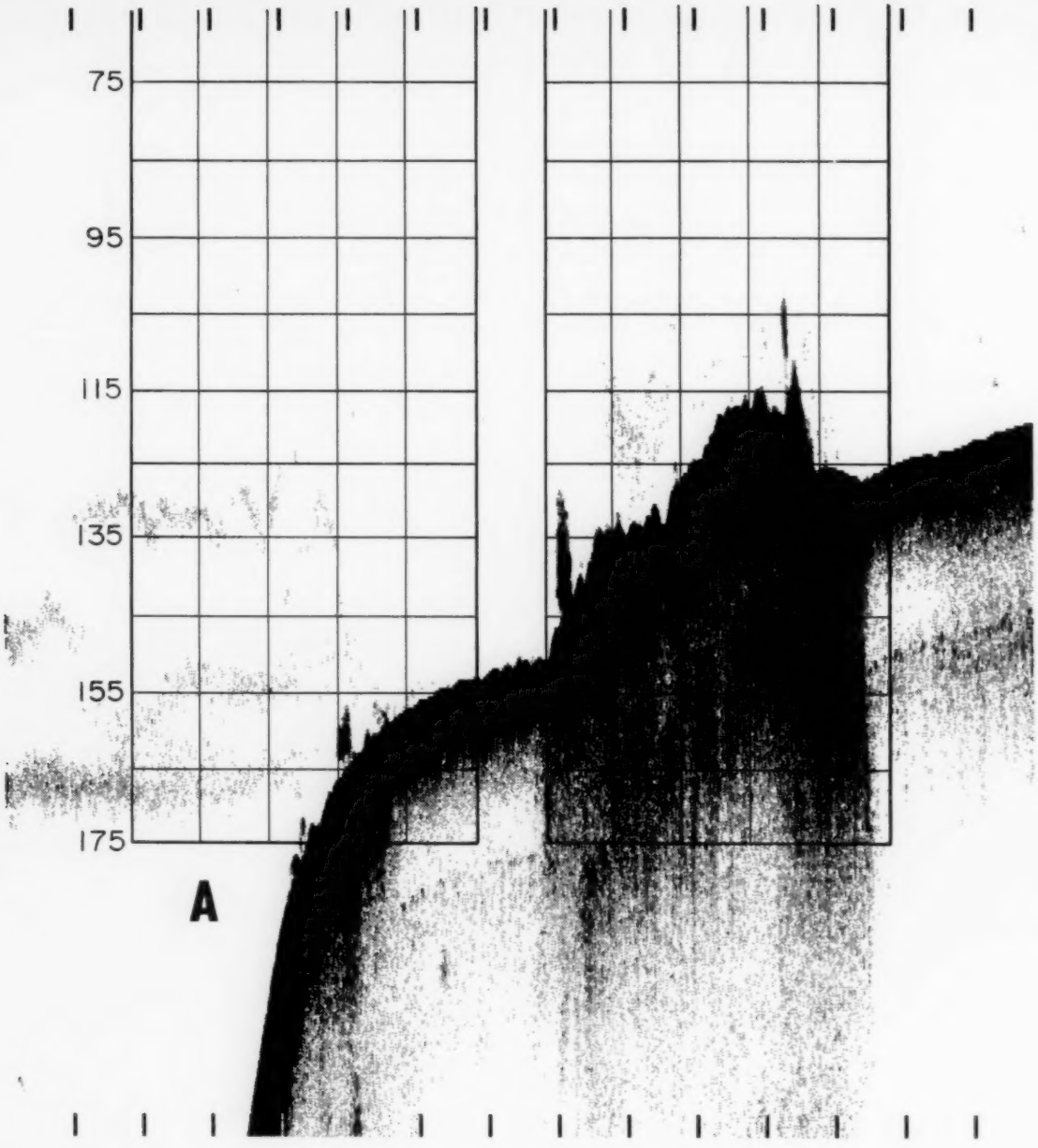

FIG. 1. Typical echograms from (A) day and (B) night transects. The dark vertical lines define the 5-min boundaries of the slope and pinnacle objects. The depth ranges from 65 to 215 m, with an integration range of 65–175 m. The fine grid denotes the integration intervals. (*Fig. 1 concluded next page*)

inating a matrix element from analysis of the volume density matrix for object k. These are given by (A) $v_{ijk} < c$, where c is a critical value defined below; (B) $v_{ijk} \geq c$, but it is apparent from echograms that plankton rather than fish have been measured; (C) $v_{ijk} \geq c$, but midwater fish schools have been measured; (D) $f_{ijk} = 0$; (E) $0 < f_{ijk} < 0.1$; and (F) integration of the bottom echo is suspected. To determine the features, we require a binary weight w_{ijk}, defined such that

$$(3) \quad w_{ijk} = \begin{cases} 0; \text{ conditions (A) – (F) are true} \\ 1; \text{ otherwise.} \end{cases}$$

Thus, (3) identifies matrix elements that represent volume densities of the bottom-oriented fish aggregations.

The critical value c is an estimate of the minimum value of v_{ijk} for fish targets. Volume densities less than c are assumed to describe background noise. To determine the appropriate

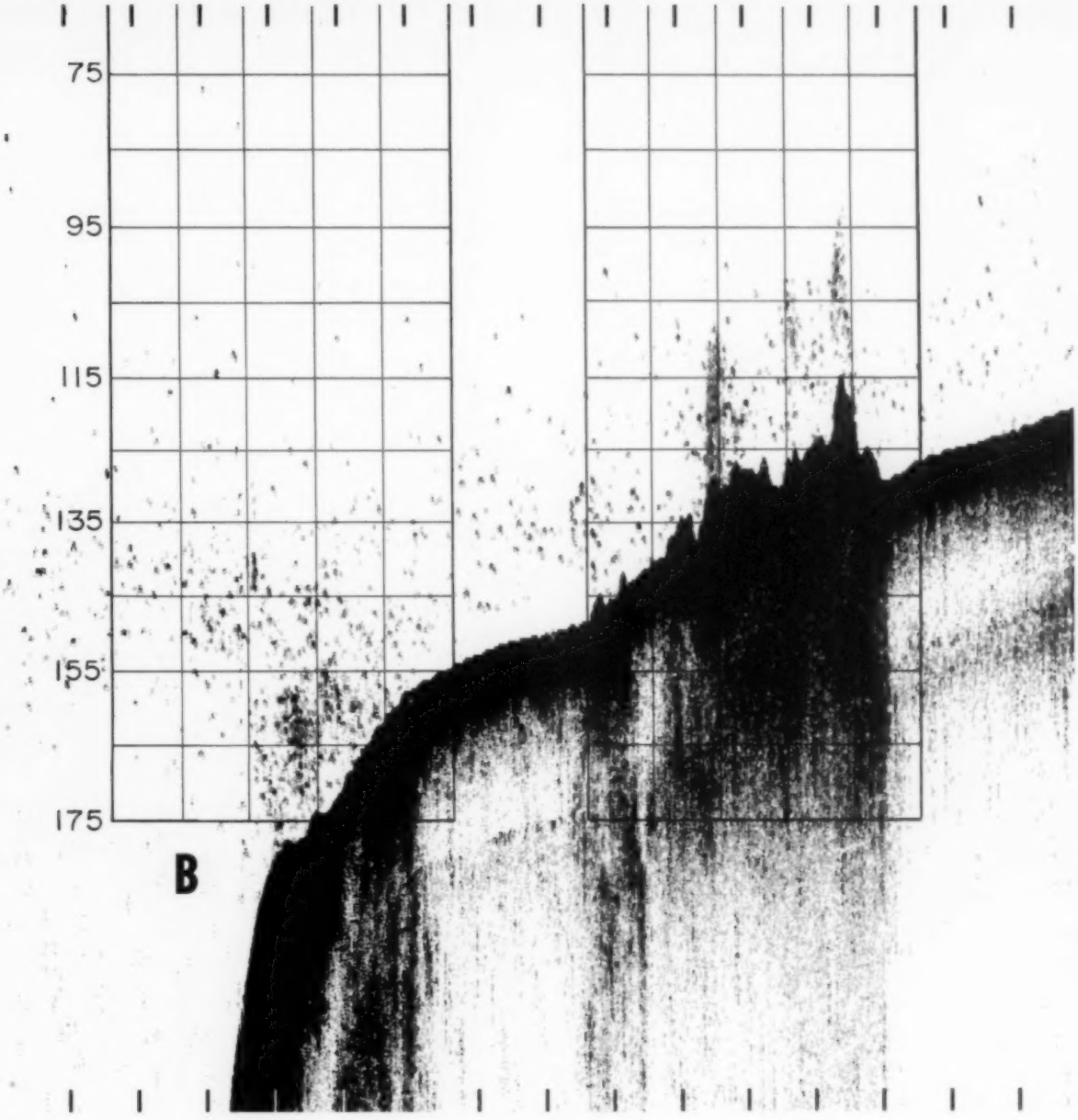

FIG. 1. (*Concluded*)

value for c, we selected 500 echogram intervals with no recognizable fish targets and an additional 500 echogram intervals with 6–30 recognizable fish targets. The range of 6–30 fish targets was used to emphasize the distinction between fish and background noise. Volume density distributions from the integration data corresponding to the two groups of intervals are illustrated in Fig. 2. There is only a small overlap in the ranges of the two groups. Based on Fig. 2, we chose a value $c = 0.02$ g/m^3, the left endpoint of the first histogram bar for which intervals representing fish schools are at least twice as frequent as intervals representing background noise.

An additional problem in interpreting volume densities is the presence of plankton. Plankton was evident from 30% of the slope objects and 14% of the pinnacle objects. Volume densities measured from the plankton concentrations are comparable with volume densities of single fish, and inclusion of these data could have confounded the results. Fortunately, plankton were most pronounced on daytime echograms, when single fish targets were rarely present. This enabled us to exclude matrix elements representing plankton concentrations without sacrificing fish data. An average of 9.1 and 2.5 elements per matrix were excluded from the analysis of slope and pinnacle objects, respectively.

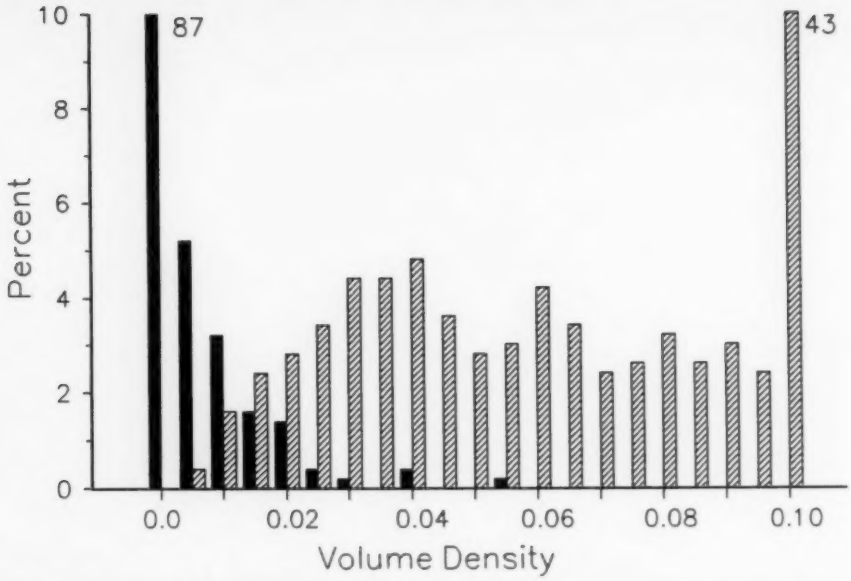

FIG. 2. Frequency distributions of the volume densities (g/m^3) of 500 integration intervals corresponding to background noise (solid bars) and 500 integration intervals corresponding to 6–30 recognizable fish targets (hatched bars). The first and last bars have been truncated and represent the percentages indicated by the bars. The last interval includes all volume densities ≥ 0.10 g/m^3.

As our purpose is to characterize fish schools associated with the bottom habitat, we also eliminated matrix elements representing pelagic fish schools. These schools, located high in the water column, are distinguished by a separation of at least one 10-m integration interval from any bottom-oriented school. Pelagic schools were identified from 49% of the slope objects and 7% of the pinnacle objects. The difference in incidence between habitat categories reflects the greater bottom depth of the slope region, and thus the greater ocean volume available to pelagic species.

If $f_{ijk} = 0$, then element ijk is located below bottom and the integrator does not report a corresponding value v_{ijk}. However, if $0 < f_{ijk} \leq 1$, then v_{ijk} reflects the density in the water volume above the bottom. We eliminated elements from the analysis if $f_{ijk} < 0.1$ to avoid extrapolating volume densities from small sample volumes. This condition occurred in 68% of the volume density matrices, with a mean of 1.8 elements eliminated per matrix.

Accurate volume density measurements are difficult near the bottom and our integrator was not always able to track the bottom profile perfectly. In particular, failure of bottom tracking was a problem over the bedrock outcrops, where depth changed rapidly. Integration of the bottom echo was identified by comparing volume densities with the corresponding echogram section. We eliminated a mean of 2.0 matrix elements with anomalously high volume densities from volume density matrices representing 41 and 9% of the pinnacle and slope objects, respectively. In these cases, fish aggregations could not be distinguished from bottom. It is possible that a few elements representing fish biomass were also excluded by this approach. However, as pinnacle objects tended to have a higher mean volume density than slope objects, the process acted to minimize density differences between the two categories.

Of the 120 available objects, six were excluded initially due to equipment malfunctions or obvious bottom integration. Nine were excluded after applying the criterion (3). For these nine objects, there were no measurable fish volume densities, i.e.

$$(4) \quad \sum_{i=1}^{n} \sum_{j=1}^{m} w_{ijk} = 0.$$

The 105 remaining objects correspond to 58 pinnacle and 47 slope regions and represent bottom-oriented fish aggregations that are typical of those recorded during the cruise.

Feature Selection

The success of pattern recognition depends to a large extent on judicious feature selection. We chose four variables from the survey data as features for NNA. These are time of day, dispersion, log mean volume density, and mean off-bottom distance. We do not consider features based on depth. Since slope habitats are all located at a depth greater than pinnacle habitats, such features would differ a priori in value between categories. Classification of fish aggregations must be based on characteristic features of the fish echo distribution, rather than on artifacts of geographic location.

The definition of time of day, T_k, is straightforward and refers to the Pacific Standard Time at which the first integration interval for object k was measured. The remaining feature definitions are determined from values v_{ijk}, f_{ijk}, and R_{jk} from the echo integrator and w_{ijk} from equation (3). For example,

$$(5) \quad N_k = \sum_{i=1}^{n} \sum_{j=1}^{m} w_{ijk}$$

is the number of matrix elements for object k meeting the criterion (3). A low value of N_k in (5) indicates that fish are

recorded for just a few of the integration intervals. Conversely, a high value indicates that fish aggregations are distributed over many of the intervals. Hence, (5) is a measure of fish dispersion. Although other dispersion measures could be defined, (5) is adequate for our analysis. In particular, $1 \leq N_k \leq 55$ for all objects, since $n \times m = 55$ and we excluded matrices for which $N_k = 0$.

Mean volume density for object k is obtained from

$$(6) \quad V_k = \exp\left[(1/N_k)\sum_{i=1}^{n}\sum_{j=1}^{m} w_{ijk} \ln(v_{ijk})\right]$$

as the geometric mean of the v_{ijk}. Density histograms suggest that the v_{ijk} are adequately described by a lognormal distribution, as shown for a similar data set by Kieser et al. (1987). In addition, a logarithmic transform is appropriate for V_k in (6), so that $\ln(V_k)$ is the feature selected to depict the mean volume density of object k. In both cases, a logarithmic transformation was chosen to ensure relatively symmetric distributions. Normality is not an assumption of NNA.

The fourth and final feature is the mean off-bottom distance of the fish biomass. The depth r_i, measured from the surface to the top of row i in the volume density matrix, is related to the row index by

$$(7) \quad r_i = 55 + 10i,$$

and

$$(8) \quad d_{ijk} = R_{jk} - r_i - 5f_{ijk}$$

gives the distance d_{ijk} to the midpoint of element ijk. Equation (8) defines d_{ijk} as the difference between mean bottom depth R_{jk} and r_i in (7). The last term in (8) is a correction to reach the midpoint of the 10-m depth interval. It accounts for the fraction f_{ijk} of the depth interval located above bottom. All matrix elements represent the same unit volume U (cubic metres) when $f_{ijk} = 1$. Otherwise, the volume is reduced by the fraction f_{ijk}. Thus, the fish biomass b_{ijk} for element ijk is given by

$$(9a) \quad b_{ijk} = Uf_{ijk}v_{ijk}.$$

For convenience, we define $U = 1$, so that (9a) simplifies to

$$(9b) \quad b_{ijk} = f_{ijk}v_{ijk}.$$

Combining (3), (8), and (9b), we obtain

$$(10) \quad D_k = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m} d_{ijk}w_{ijk}\ln(b_{ijk})}{\sum_{i=1}^{n}\sum_{j=1}^{m} w_{ijk}\ln(b_{ijk})}$$

as the weighted mean off-bottom distance of the fish biomass associated with object k. Again, a logarithmic transformation is used, as the distribution $\ln(b_{ijk})$ is approximately normal.

The four features define a vector

$$(11) \quad \boldsymbol{\theta}_k = (D_k, N_k, T_k, \ln(V_k))$$

corresponding to the coordinates of a point k in four-dimensional feature space. Thus, (11) describes a transformation from a matrix of fish volume densities, depths, etc., to a four-dimensional feature space in which each point $\boldsymbol{\theta}_k$ represents an object. To identify which, if any, of the features discriminate between habitat categories, we first examine selected

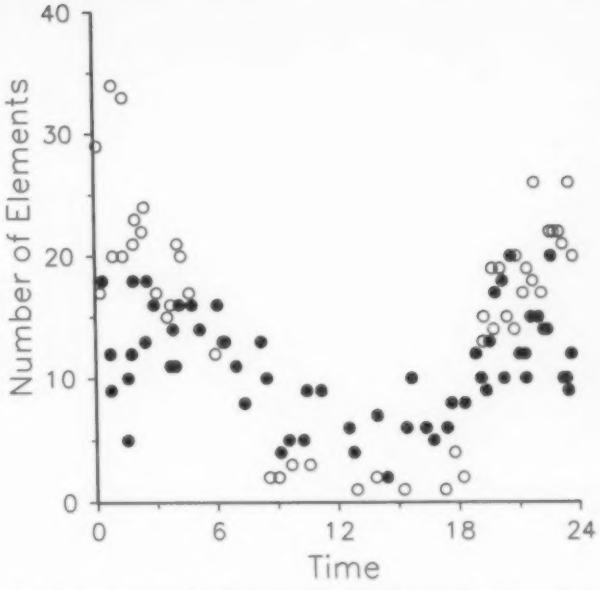

FIG. 3. Scatter plot of the dispersion (elements) in equation (5) against time (h) for pinnacle (solid circles) and slope (open circles) objects.

feature pairs in two-dimensional scatter plots. We do not attempt to remove the correlations among features because we wish to retain their physical interpretation. We drop the subscript k in reference to the features D, N, T, and $\ln(V)$, as opposed to the particular value for object k.

Prior to applying NNA, all features were standardized to a mean 0 and variance 1. Standardization is necessary to ensure that the units of measure for each feature do not influence the calculated distances between pairs of data points. The analysis was conducted with a Euclidean distance metric, using the algorithm provided by the SAS software package (SAS Institute Inc. 1985). The distance between two points x and y is given by

$$(12) \quad S_{xy} = |\boldsymbol{\theta}_x - \boldsymbol{\theta}_y|.$$

If a subset of the vector (11) is to be considered, then only the coordinates of interest are retained in (12).

Because time is a circular rather than linear variable, a modification to the procedure was required for analyses involving T. For example, the nearest neighbour to a point with a value $T = 23.8$ h could have a value $T = 0.2$ h, but the algorithm would incorrectly select the neighbour with a value $T = 23.0$ h. To overcome this difficulty, we formed two additional copies of the data set. For one of these copies, 24 h was added to each value of time, and for the other copy, 24 h was subtracted from each value of time. The analysis was then conducted using all points with a value of time in the range (−12 h, 36 h) and classification success was scored for the original data only, with values T in the range (0 h, 24 h).

Results

Typical night and day echograms are illustrated in Fig. 1. Near the shallow end of the transect, the bottom topology is characterized by a series of bedrock outcrops that create local relief, up to 30 m in height, and are interspersed with small

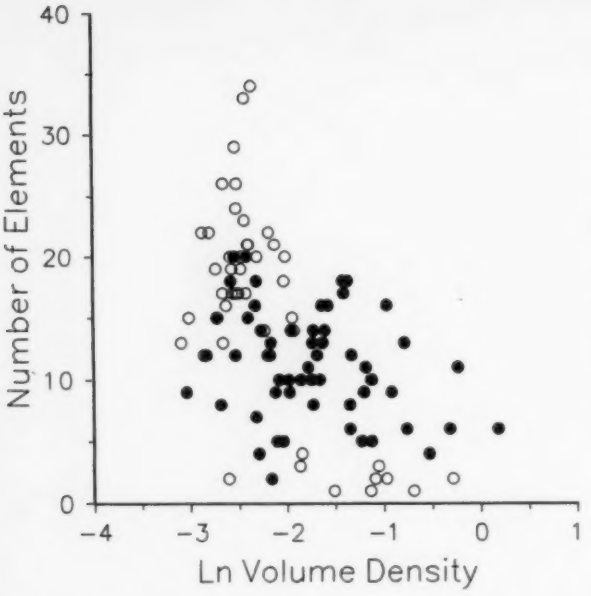

FIG. 4. Scatter plot of the dispersion (elements) in equation (5) against mean volume density (g/m^3) in equation (6) on a logarithmic scale for pinnacle (solid circles) and slope (open circles) objects.

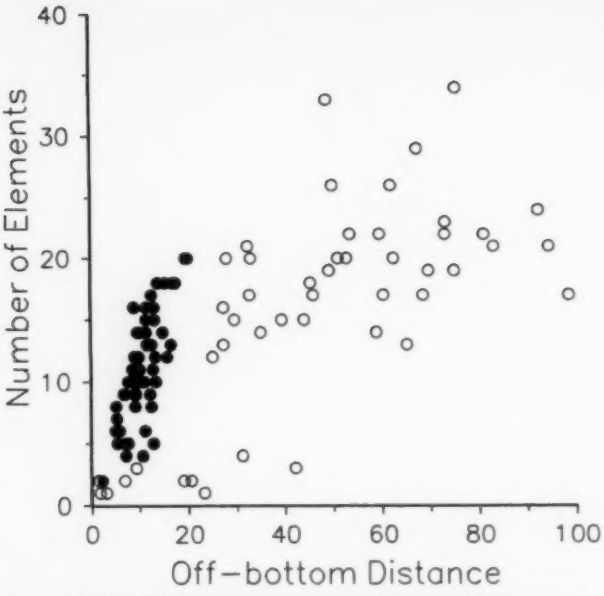

FIG. 5. Scatter plot of the dispersion (elements) in equation (5) against mean off-bottom distance (m) in equation (10) for pinnacle (solid circles) and slope (open circles) objects.

basins (Bornhold and Yorath 1984). The bottom appears relatively smooth over the remainder of the transect. The continental shelf break occurs near a depth of 170 m and is associated with an abrupt change in the bottom slope. There are tall, columnar fish aggregations over the bedrock outcrops during the day and smaller aggregations adjacent to the shelf break. We believe that the light markings extending from the shelf break represent plankton. By contrast, during the night, fish appear dispersed, with denser aggregations near the bedrock outcrops and shelf break. The day/night transition occurs within a 0.5-h period near dusk and dawn. We selected the four features D, N, T, and $\ln(V)$ in an attempt to quantify this behaviour.

A scatter plot of dispersion N against time T (Fig. 3) illustrates some of the day/night differences in the fish aggregations. The greatest dispersion occurs near midnight and the lowest near midday. This pattern is more pronounced for slope than pinnacle objects. In general, N is greater for slope objects during the night, with the reverse being true during the day. In fact, nine daytime slope objects were excluded during preliminary processing by the criterion (4). For the remaining slope objects, N is near 0 during the day.

Differences between habitat categories are also evident in Fig. 4, a scatter plot of N against $\ln(V)$. The nighttime slope objects with a high value N in Fig. 3 correspond to a low value $\ln(V)$. Conversely, the daytime slope objects with a low value N in Fig. 3 tend to have a high value $\ln(V)$. This pattern mirrors echogram observations in that slope aggregations disperse at night and congregate during the day. The inverse relationship between N and $\ln(V)$ for slope objects may indicate a consistency in the biomass of slope fish from day to night, with simply greater dispersion during the night. There is no apparent relationship between N and $\ln(V)$ for pinnacle objects. Thus, pinnacle fish aggregations do not tend to vary systematically in their dispersion and density. The higher values $\ln(V)$ for pinnacle objects at intermediate values N improve the separation by habitat category over that shown in Fig. 3.

TABLE 1. Percentage of the 105 objects correctly classified by NNA, using L = 1, 3, 5, and 7 nearest neighbours, for combinations of mean off-bottom distance D, dispersion N, time T, and mean volume density V. A natural logarithmic transform was applied to V, and all variables were standardized to a mean of 0 and variance of 1 prior to the analysis. An asterisk indicates that the results are not significantly different from a binomial distribution (with a probability $p = 0.5$) at the 99% probability level.

	Number of neighbours (L)			
Variables	1	3	5	7
(D,N)	97	97	97	96
(D,T)	93	91	92	90
(D,V)	91	92	92	92
(N,T)	76	78	79	71
(N,V)	81	82	83	82
(T,V)	54*	58*	61*	66
(D,N,T)	94	93	93	92
(D,N,V)	97	94	91	90
(D,T,V)	89	89	91	90
(N,T,V)	77	82	83	86
(D,N,T,V)	93	93	88	88

Mean off-bottom distance D is distinctly different for the pinnacle and slope objects (Fig. 5). For pinnacle objects, D is correlated with N ($r^2 = 0.57$). No pronounced relationship is apparent for slope objects. In fact, most values D for slope objects exceed the maximum D for pinnacle objects. Although the ocean bottom drops off steeply in the region of the slope, these large differences in D are not simply an artifact of the data processing. The underestimate of bottom depth, a problem with the calculation of d_{ijk} in (8), leads to lower values D for slope objects than would otherwise be obtained. Hence, true

differences in D between habitat categories are more extreme than illustrated in Fig. 5. Furthermore, it is evident from nighttime echograms that slope fish aggregations form a band extending horizontally from the slope, with portions of the school in midwater. This behaviour is markedly different from that of the pinnacle aggregations, which tend to maintain a close proximity to the bottom profile. The distribution of points in feature space has quantified this behavioural phenomenon.

Table 1 includes the results of NNA using L values of 1, 3, 5, and 7 neighbours for all possible combinations of features in the vector (11). Distinctions between pinnacle and slope objects are pronounced in Fig. 5, and indeed, the feature pair (D,N) produces the highest classification success (97%). Other two-feature combinations involving D provide a classification success of 90–93%. Clearly, mean off-bottom distance is the most useful discriminator for these data. Even without the feature D, classification success of up to 83% can be achieved with the pair (N,V). In fact, only the pair (T,V), with a classification success of 54–66%, did not produce useful discrimination. The success rate must be greater than 61% to differ from chance at the 99% probability level, based on the binomial test (Siegel 1956).

The three- and four-feature combinations in Table 1 provide slightly poorer classification success than expected on the basis of the corresponding two-feature combinations. The one exception is (N,T,V) which leads to a higher classification success (86%, $L = 7$) for one value of L than (N,V), the best corresponding two-feature subset. The general deterioration in classification success with increasing numbers of features is characteristic of NNA, when the additional features have less information on among-category differences than the initial features. Since each feature receives equal weight in the analysis, extra information can act to introduce noise. Consequently, the addition of a third feature cannot be justified for these data.

A criticism of our analysis might involve the definition of objects. To verify the robustness of our results, we repeated the NNA with an alternative object definition. We selected 3-min rather than 5-min echogram sections. The deepest 2-min sections were deleted from slope objects and pinnacle objects were selected by centering 3-min sections over the bedrock outcrops. Classification success was poorer with this analysis, as expected from the use of fewer data in defining the features and the reduced depth range for slope objects. However, feature pairs were more similar in their relative success. Except for (T,V), all two-feature combinations led to a classification success of 86–87%. This represents an improvement in the success of pairs (N,T) and (N,V) and a decrease in the success of pairs (D, N), (D, T), and (D, V). In addition, the classification success of the pair (T,V) improved to 67–69%.

Discussion

The type of echo integration data we describe here is routinely collected during hydroacoustic biomass surveys. Our analysis has shown such data to have wide utility. In addition to providing biomass estimates, these data can be used to classify fish assemblages with a high degree of accuracy. We obtained up to 97% correct classification of pinnacle and slope fish assemblages using mean off-bottom distance and a measure of dispersion. A classification success of 93% was obtained with off-bottom distance and time of day, and a classification success of 83% was achieved with dispersion and mean volume density. These results compare favourably with 93% correct classification of cod and capelin schools reported by Rose and Leggett (1988), based on detailed analysis of the acoustic echo.

A fishing vessel operated in close proximity to the hydroacoustic vessel. Fishing results are reported by Matthews et al. (1989). Using sunken gill nets, six and four sets were completed in the pinnacle and slope regions, respectively. Different rockfish species dominated the gillnet catch in the two regions. The major species, by number, in the pinnacle region were yellowtail rockfish, *Sebastes flavidus* (45%); bocaccio, *S. paucispinis* (32%); and canary rockfish *S. pinniger* (16%) whereas in the slope region, the major species were sharpchin rockfish, *S. zacentrus* (27%); widow rockfish, *S. entomelas* (19%); redbanded rockfish, *S. babcocki* (11%); and silvergray rockfish, *S. brevispinis* (11%). The species composition was consistent with trawl surveys (Gillespie and Stanley 1989; Stanley 1988) and commercial catches from the same area (Nagtegaal 1983).

Rockfish depth and habitat specificity is well known from both trawl surveys (Chen 1971; Gabriel and Tyler 1980; Westrheim 1975) and direct visual observations (Hallacher and Roberts 1985; Richards 1986). Thus, differences in species composition between the pinnacle and slope assemblages are not unexpected. There are few publications, however, that report hydroacoustic observations on rockfish. The most extensive study was conducted by Wilkins (1986), who described schools of widow rockfish as tall, slender columns present over irregular bottom, with smaller schools high in the water column in the evening and early morning. Schools tended to grow in size as the night progressed. Such a description could apply to many of the schools we observed. Indeed, Wilkins (1986) mentioned two other rockfish species that appear similar to widow rockfish on echograms. Determination of rockfish species comprising specific schools is clearly a difficult task, although hydroacoustic methods show some promise in this area.

The goal of our feature selection was to quantify patterns in the fish behaviour illustrated on the echograms, and thus to classify them correctly into habitat categories. Diel changes in rockfish behaviour facilitated the choice of features and the performance of our methods. However, as fish behaviour and the species composition of the assemblages may change seasonally, we do not expect the choice of features to be consistent among studies conducted at different times or in different areas. Our methods are intended to act as guidelines for examining hydroacoustic data rather than as a recipe for rockfish species recognition.

Our pattern recognition problem was atypical in one respect. Usually, the observations for an individual entity comprise a vector with each component representing a different phenomenon. For example, the vector could consist of length, weight, and sex measures for each entity. In our case, the individual objects were represented not by a vector, but by a matrix of volume densities and associated ancillary information. With the exception of time, the features corresponded to statistical properties of the matrix elements.

Our study was not intended to design the optimum algorithm for fish species recognition. Instead, we demonstrated the species discrimination capability inherent in typical echo integration data. Another application of our methods might be hydroacoustic biomass surveys for Pacific hake, *Merluccius productus*. The presence of Pacific herring, *Clupea harengus pallasi*, is a problem during these surveys, and classification of fish schools is determined by visual inspection of the echograms with some concurrent fishing. NNA could be used to classify schools on echograms as herring or hake, and thus

improve the hake biomass estimates. In general, much more information is contained in the hydroacoustic data than has traditionally been used. With the advent of computer processing, such additional information may prove to be as valuable as the biomass distribution, the current end product of many acoustic surveys.

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Adaptive Management of Spatially Replicated Groundfish Populations

Jeremy S. Collie[1] and Carl J. Walters

Resource Ecology, The University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

Collie, J. S., and C. J. Walters. 1991. Adaptive management of spatially replicated groundfish populations. Can. J. Fish. Aquat. Sci. 48: 1273–1284.

Assessment techniques that recognize information shared among substocks can help reduce uncertainty about stock size and productivity, compared with methods that treat each subpopulation independently. We develop this methodology using time series of catch and relative abundance data for six subpopulations of yellowtail flounder, *Limanda ferruginea*, from the Northwest Atlantic. The Deriso/Schnute delay-difference production model was fit to the data, but the model parameters associated with population productivity and absolute population size were very uncertain. Estimates of parameter uncertainty were used to identify a set of models that are consistent with the observed data and imply different management policies. The value of these different policies was estimated with a stochastic simulation program that uses a Kalman filter to predict stock size for each model and Bayesian updating to assign a probability of each model being correct. With parameters shared among stocks and a shared response to external influences, in the order of 10–20 yr was needed to distinguish the correct model. Adaptive management simulations indicated that long-term yield was greater in simulations with adaptive learning and that a lower initial exploitation rate should be applied than would be the case if adaptive learning were ignored.

Les techniques d'évaluation qui permettent de distinguer les caractéristiques communes à certains des sous-groupes d'un stock peuvent contribuer à réduire le degré d'incertitude concernant l'effectif et la productivité d'un stock à la différence des méthodes dans lesquelles on traite chaque sous-population séparément. Nous avons mis au point la méthode présentée ici en utilisant des séries chronologiques de données sur les prises et l'abondance relative de six sous-populations de limandes à queue jaune, *Limanda ferruginea*, du nord-ouest de l'Atlantique. Nous avons pu ajuster le modèle de production à différence de délai, mais les paramètres associés à la productivité et à l'effectif absolu de la population comportaient un grand degré d'incertitude. Nous avons employé des estimations du degré d'incertitude paramétrique pour constituer un ensemble de modèles concordant avec les observations et supposant différentes politiques de gestion. Nous avons évalué ces différentes politiques au moyen d'un programme de simulation stochastique dans lequel un filtre Kalman sert à prévoir l'effectif des stocks en fonction de chaque modèle et nous nous sommes servi de la méthode de mise à jour bayesienne pour attribuer une probabilité de justesse à chacun des modèles. Avec les paramètres communs à plusieurs stocks et une réponse commune aux influences externes, il a fallu aux alentours de 10 à 20 ans pour reconnaître le modèle juste. L'étude de simulations de gestion adaptative a indiqué que la productivité à long terme était supérieure dans les simulations à apprentissage adaptatif et qu'il fallait que l'exploitation soit au début plus lente qu'elle ne le serait si l'on ne tenait pas compte de l'apprentissage adaptatif.

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Many species of groundfish exist as spatially discrete subpopulations and are assessed and managed as separate stocks. They share population parameters and respond similarly to coastwide environmental fluctuations (Koslow et al. 1987). By recognizing these linkages among populations, fisheries scientists may be able to improve the estimation of shared population parameters and to use this information to better manage the stocks. Management policies may be designed to trade off the catch among subpopulations in ways that would enhance learning about the population parameters, while keeping the total catch relatively stable. The purpose of this paper is to develop methods for estimating the value of such adaptive policies and to illustrate these methods with a case study of the yellowtail flounder, *Limanda ferruginea*.

The rationale of adaptive management is to consider fishing as an experiment and to use the results of experimentation to increase long-term yield or return from the fishery (Walters 1986). Until recently, adaptive management policies have been applied only to single stocks, mainly salmonids (e.g. Walters and Hilborn 1976; Collie and Walters 1987). Adaptive management is difficult to implement because it often requires short-term pain for long-term gain: current yields must be sacrificed in the quest for larger long-term yields. Applied to single stocks, adaptive policies are poor experiments because there are no replicate or control stocks. Even if the population responds as expected, one cannot be sure that the stock responded to harvesting or to some other, perhaps environmental, trend.

These problems can be at least partly avoided by experimenting on spatially replicated stocks. The "replicate" subpopulations are not quantitatively identical but should respond similarly to fishing pressure and environmental perturbations.

[1]Present address: Juneau Center for Fisheries and Ocean Sciences, University of Alaska, Juneau, AK 99801, USA.

Information gained on one subpopulation should apply to some extent to other subpopulations. Then, trading off catch among subpopulations, it should be possible to ease the fisherman's short-term pain of implementing adaptive policies. Following Gulland (1976), we distinguish between a species population and a fisheries stock by defining the stock as an operational unit with homogeneous demographic parameters. Subpopulation is synonymous with stock.

There are two recent examples of adaptive management policies applied to replicated groundfish populations: the multispecies fishery off the northwest coast of Australia (Sainsbury 1988) and the British Columbia fishery for slope rockfish (Walters and Collie 1989). A big difficulty in both studies has been how to determine the amount of information shared among subpopulations. In the first case, subpopulations were assumed to be identical; in the second, they were assumed to have completely independent recruitment parameters and environmental effects and to share only growth and survival parameters. In the present paper, subpopulations are assumed to share information on some, but not all, population parameters and to exhibit shared responses to environmental factors. In particular, we assume a common response of recruitment at low stock sizes (susceptibility to recruitment overfishing) and some shared variation around stock-specific recruitment curves.

The steps involved in an adaptive analysis of an exploited population are as follows: (1) identify a range of alternative hypotheses (models of population dynamics) consistent with the historical data that make unique predictions about the response to harvesting; (2) establish a model of how future data will be gathered and used to discriminate among the alternative hypotheses; (3) identify a range of management policies (harvest rates); and (4) calculate the expected value (e.g. discounted sum of future catches) of each policy given the uncertainty about which hypothesis is correct. The following sections demonstrate these steps, as applied to the yellowtail flounder, for which fairly complete data exist for a number of subpopulations. The types of catch and abundance data used in this example are routinely collected in many fisheries. Although we hope that the policies identified in this paper will be useful for managing yellowtail flounder, we are concerned less with the management of yellowtail flounder per se than with the applicability of adaptive management policies to groundfish in general.

Historical Data Base

The yellowtail flounder is a commercially important flatfish along the east coast of North America from Labrador to Chesapeake Bay (Fig. 1) at depths of 10–100 m (Lux and Livingstone 1982). We compiled catch and abundance data on six subpopulations of yellowtail flounder from the following sources: Grand Banks (Brodie 1986); Scotian Shelf (Neilson and Perley 1986); and Cape Cod, Georges Bank, Southern New England, and Mid Atlantic (Clark et al. 1984). The northern subpopulations (Grand Banks and Scotian Shelf), being separated by deep channels, are spatially discrete. Although there are no significant morphometric differences among the New England stocks, there appears to be little mixing among them, at least at the adult stage (Royce et al. 1959). Tagging studies indicate limited migration (less than 5% of recovered tags) between Southern New England and Georges Bank but no mixing of these stocks with the Cape Cod subpopulation (Lux 1963).

The largest yellowtail flounder stocks are found on the Grand Banks, Georges Bank, and off Southern New England (Fig. 2). The catch histories of these stocks are similiar: large catches were taken in the late 1960s and early 1970s, catches were low in the late 1970s, and increased slightly in the early 1980s. The are two sources of relative abundance data: the commercial catch-per-unit-effort (CPUE) index and standardized research trawl survey data. The CPUE data are less varible than the survey data, presumably because the former are based on larger sample sizes. The spikes and troughs in the survey data do not correspond to actual changes in population biomass; such rapid changes are inconsistent with age-structured catch and abundance data.

Instead, the variability of the survey data is due to changes in the distribution and aggregation of the fish in relation to the survey strata and/or to variability in trawl success. Whatever causes the variability in the survey data, the deviations in abundance are often consistent among subpopulations. For example, note the positive deviations in abundance in 1972 for Cape Cod, Georges Bank, Southern New England, and Mid Atlantic and the positive deviations in 1977 for the Grand Banks, Scotian Shelf, and Cape Cod stocks. The fact that these deviations are correlated among subpopulations should make it easier to discern the underlying population dynamics than would be the case if the deviations were uncorrelated.

Despite the variability of the survey data, the two types of relative abundance data show roughly the same trends: abundance was high in the early 1970s, decreased in the late 1970s, and increased modestly in the early 1980s. There are at least two plausible explanations of these tends: abundance decreased due to overfishing in the early 1970s or abundance decreased due to some coastwide environmental factor. Because the subpopulations have similar catch histories, it is difficult to distinguish the effects of fishing from possible environmental influences. This problem is particularly acute in marine fish populations for which we cannot measure population size directly but only indirectly through catch indices. Clearly, any model of population dynamics must include a proportionality constant to scale relative abundance to absolute catch.

Delay-Difference Model

The population model we fit to the yellowtail flounder data is the delay-difference equation as derived by Deriso (1980), generalized by Schnute (1985), and implemented by Walters (1987). The delay-difference equation provides a simple way to describe the dynamics of an exploited population with biologically realistic parameters. With this equation, it is possible to account for biomass growth without keeping track of the numbers of fish in each age class. Even if catch-at-age data are available, a biomass-based model may be preferable if aging errors are substantial. The main reasons for choosing the delay-difference equation over other candidate models are that it is applicable to both types of relative abundance data for all subpopulations and it can readily be used to simulate forward in time.

According to the delay-difference equation, next year's biomass (B_{t+1}) is the sum of the biomass (P_t) remaining after a discrete catch (C_t), adjusted for natural survival (s_t) and body growth, plus next year's recruitment (R_{t+1}):

$$(1)\quad B_{t+1} = s_t(\alpha/w_t + \rho)P_t + R_{t+1}.$$

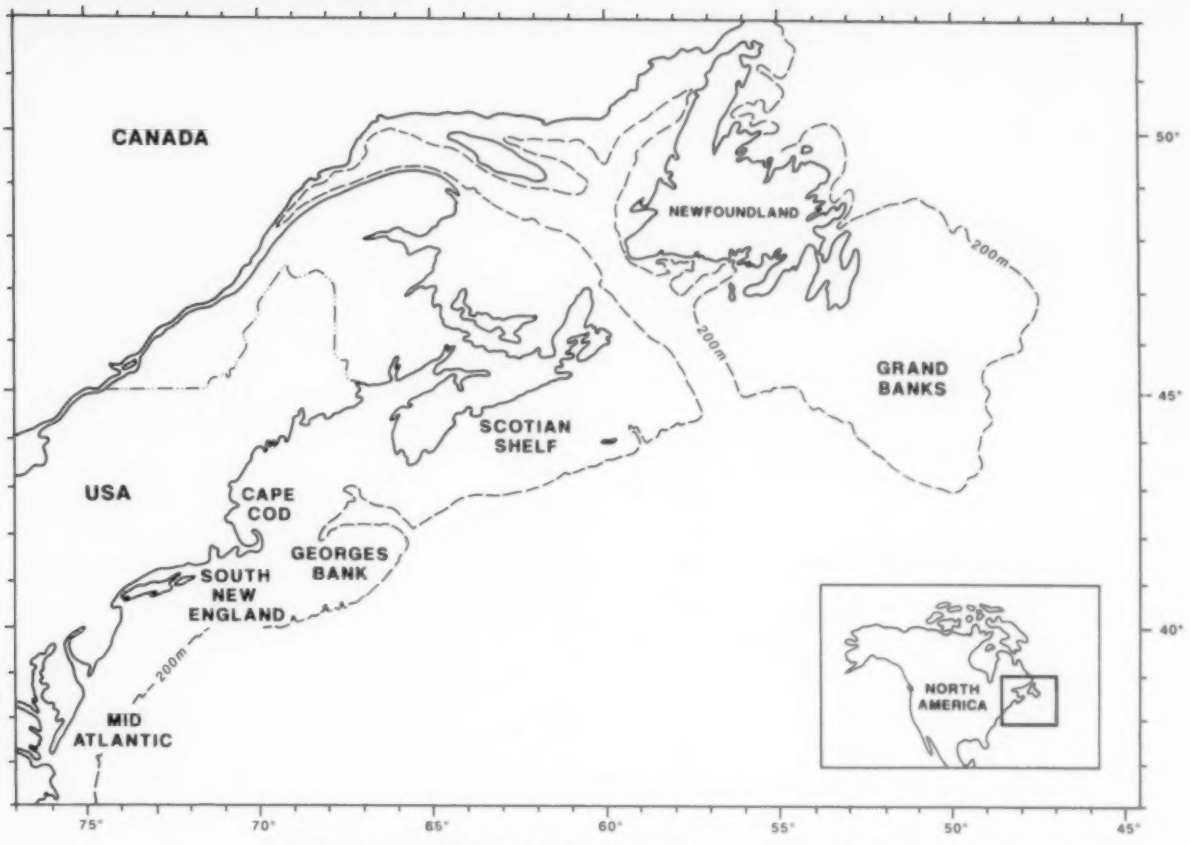

FIG. 1. Western North Atlantic with the major stocks of yellowtail flounder.

Natural survival (s_t) is assumed to be independent of age but may vary with time. Body growth is modelled by the Ford growth equation:

$$(2) \quad w_j = \alpha + \rho w_{j-1}$$

where w_j is weight at age j and the parameters α and ρ are fitted with a Walford plot. For most exploited fishes, the annual growth increment decreases linearly with increasing body weight ($w_j - w_{j-1} = \alpha + (\rho - 1)w_{j-1}$) and the constant ρ is about 0.8.

The model assumes that the fish mature at the same age they recruit to the fishery but that recruitment of a cohort may be spread over several years. Recruitment (R_t) is thus a sum of the progeny (Y_{t-j}) of previous spawning stocks (P_{t-j}) weighted by the proportion maturing at each age. The progeny, in biomass units, is assumed to be some function of parental biomass, and in this case we used the Ricker equation:

$$(3) \quad Y_t = P_t \exp(a - bP_t).$$

If mean weight (w_j) in Eq. 1 is unknown, it can be replaced by a second-order difference term such that the equation actually fit to the data is

$$(4) \quad B_{t+1} = s(1+\rho)P_t - s\rho(P_t/B_t)(sP_{t-1} + vR_t) + R_{t+1}.$$

The ratio P_t/B_t is 1 minus the exploitation rate and v is w_{k-1}/w_k where k is the first age recruiting to the fishery.

In this application, natural survival (s) is assumed to be constant among subpopulations and among years. As the natural survival rate is generally not estimable from fisheries data, we set $s = 0.82$ ($M = 0.2$), the value commonly assigned to this species (Anonymous 1989). We estimated the growth parameters $\rho = 0.8$ and $v = 0.48$, from a Walford plot generated using data from Georges Bank (Grosslein et al. 1980) and applied these values to all of the subpopulations.

The partial recruitment vectors were determined from published records (Anonymous 1989) and from catch-at-age analyses. For the Mid Atlantic, Southern New England, Georges Bank, and Cape Cod stocks, we set the proportion recruiting at ages 1–3 at 0.25, 0.5, and 0.25, respectively. Yellowtail flounder on the Grand Banks recruit to the fishery at older ages (Brodie 1986); for the Grand Banks and Scotian Shelf stocks, we set the proportions recruiting at ages 4 and 5 at 0.5 each. These assumed partial recruitment vectors may slightly underestimate the true mean age at recruitment. If J is the maximum age at recruitment, the first J years of data are needed to predict the first recruitment and are not used for parameter estimation (Fig. 3). Often it is better to set a low J (or to assume knife-edge recruitment) than to use fewer observations in the estimation (Walters 1987). The results of Monte Carlo simulations indicate that simple production models with 1-yr lag to recruitment may perform better than age-structured models, even if the data are generated with ages to recruitment greater than 1 (Ludwig and Walters 1985). Underestimating the mean age at

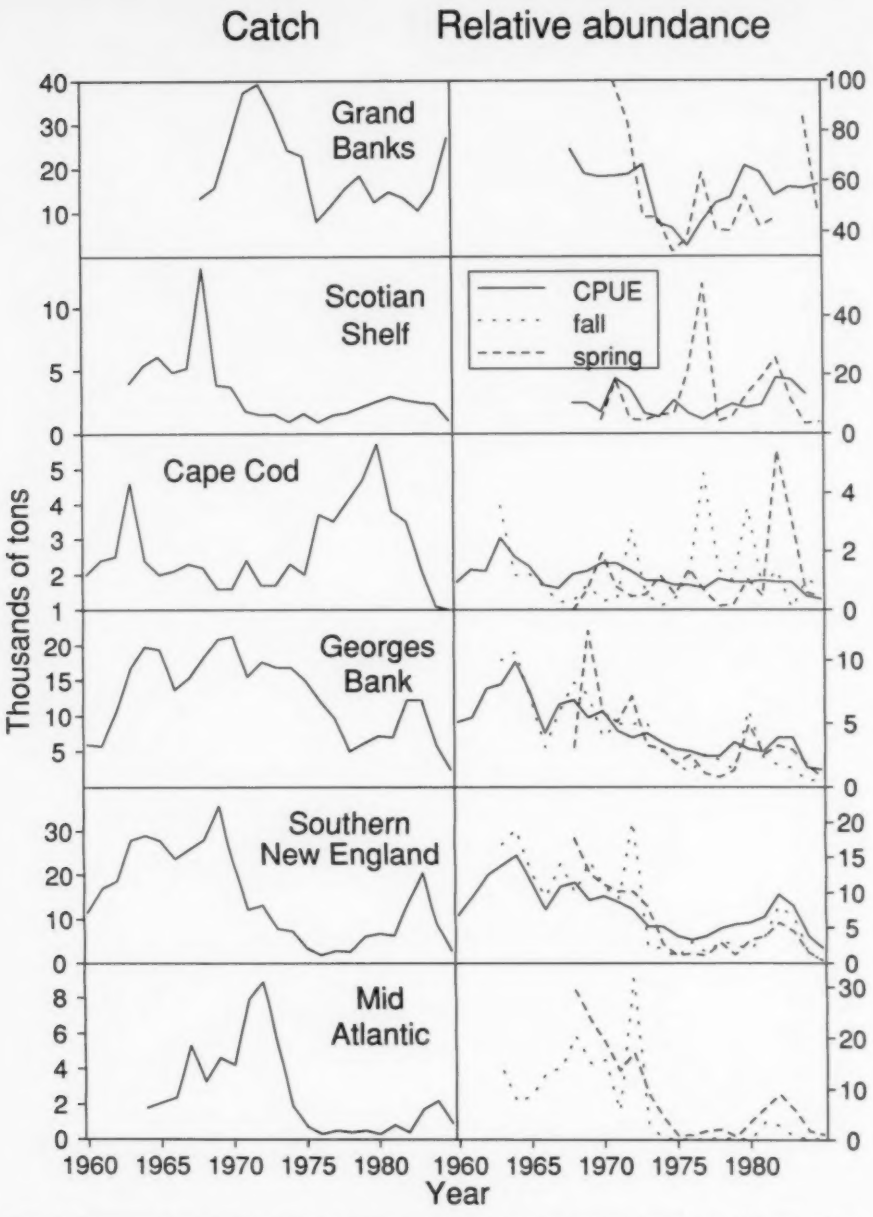

FIG. 2. Commercial catch and relative abundance of six stocks of yellowtail flounder. CPUE was scaled to be in the same arbitrary units as the fall and spring survey data.

recruitment may thus improve the parameter estimates obtained with the delay-difference equation.

We used the commercial CPUE index (Fig. 2) as the main relative abundance data and the survey index as auxiliary data. These abundance indices were assumed to be equally reliable, and each observation was assigned equal weight in fitting the delay-difference model. Relative abundance is assumed to be linearly related to population biomass, e.g. $\text{CPUE}_t = qB_t + \omega_t$ where the proportionality constant q is a catchability coefficient and ω_t is a measurement error. While it is possible to specify a nonlinear relationship between CPUE and biomass, the shape parameter usually cannot be estimated from the data, in addition to the other population parameters, and must be determined independently. The BASIC computer program GENEST fits the delay-difference model to observed time series of relative abundance data by nonlinear regression using the Newton method with step-size corrections (Walters 1987). In fitting the model with GENEST, we assumed that all error is measurement error. The parameters to be estimated are the Ricker a and b parameters and the catchability coefficient q. There is an additional q for the survey data; we actually estimate the ratio of the two q values.

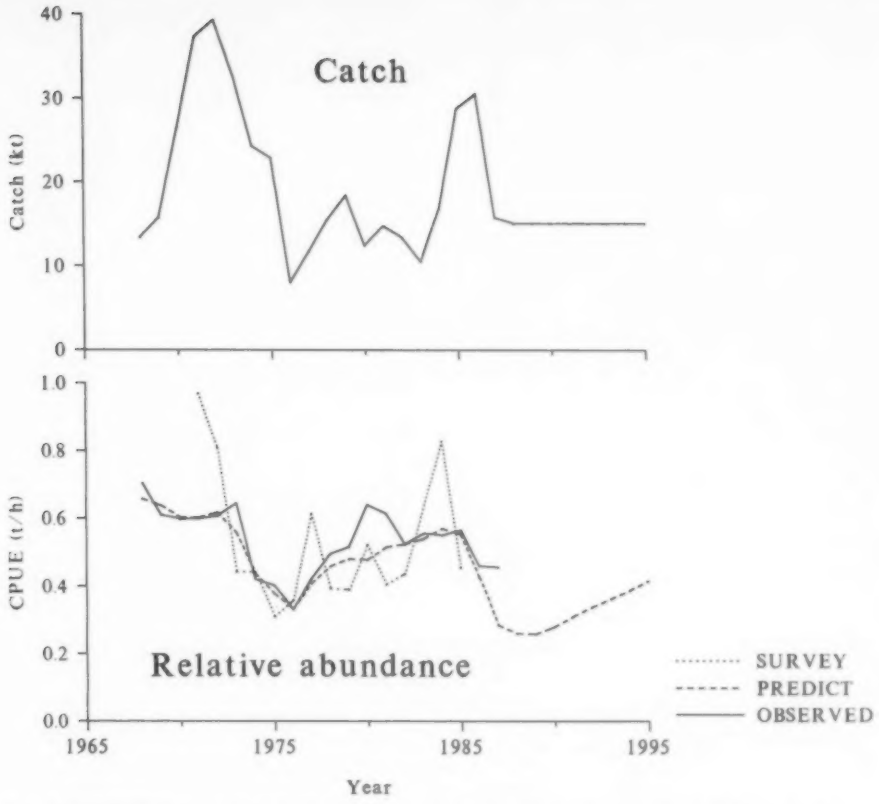

FIG. 3. GENEST fit to catch and relative abundance data for Grand Banks yellowtail flounder. Only the catch and relative abundance data up to 1985 were used to fit the model. The survey data were scaled to the same units as CPUE.

Identifying Alternative Hypotheses

The delay-difference model was fitted to data from the six subpopulations of yellowtail flounder (Fig. 2). For most stocks the auxiliary survey data helped to constrain the parameter estimates. In one case, Cape Cod, the extremely variable survey data did not help stabilize the parameter estimates, and we used only the CPUE data for this subpopulation. In this section we illustrate the results for the Grand Banks stock only; similar results were obtained for the other five stocks.

The delay-difference model fits the CPUE data quite closely (e.g. Fig. 3). The Grand Banks subpopulation was simulated beyond 1985 by removing the observed catch in 1986–87 and an annual total allowable catch (TAC) of 15 thousand metric tons (kt) from 1988 to 1995. The decline in predicted relative abundance is consistent with the decline in CPUE observed in 1986–87 and demonstrates the value of the delay-difference equation for simulation.

Although observed and predicted abundance agree quite closely, the parameter estimates are very uncertain. There are many different parameter combinations (a, b, q) that give almost identically good fits to the data. These parameter combinations represent alternative hypotheses about the population dynamics of yellowtail flounder. For example, the stocks could be small and productive (high a, b) or large and unproductive (low a, b); both hypotheses are consistent with the observed relative abundance data but imply different optimal harvest rates.

The uncertainty of the parameter estimates is depicted by the joint confidence region which is a three-dimensional volume shaped like a flattened cigar. Sections through this volume were obtained with a commonly used linear approximation (Bard 1974, p. 188) by holding one parameter at its best estimate and varying the other two (Fig. 4). The linear approximation of the confidence region is only valid in the region around the best estimate for which the sum of squares is a quadratic function of the parameters; the nonlinear confidence region is actually banana shaped. The confidence region indicates that while the parameters are very uncertain, only some combinations are consistent with the data (if one of the parameters is set, the other two are constrained within narrower limits). We identified a discrete set of parameter combinations along the major axis of the confidence region by choosing a set of a values (0, 0.3, 0.6, 0.9, 1.2) centered on the best estimate and refitting the b and q parameters. This resulted in five discrete hypotheses or models, each one represented by a different value of the Ricker a parameter, which measures productivity at low stock size. The a parameter was arbitrarily chosen as "leading parameter" because it generates a hypothesis set with obvious implications for productivity and sustainable yield.

The same set of a values was used to generate alternative hypotheses for each yellowtail flounder stock. Generally, the

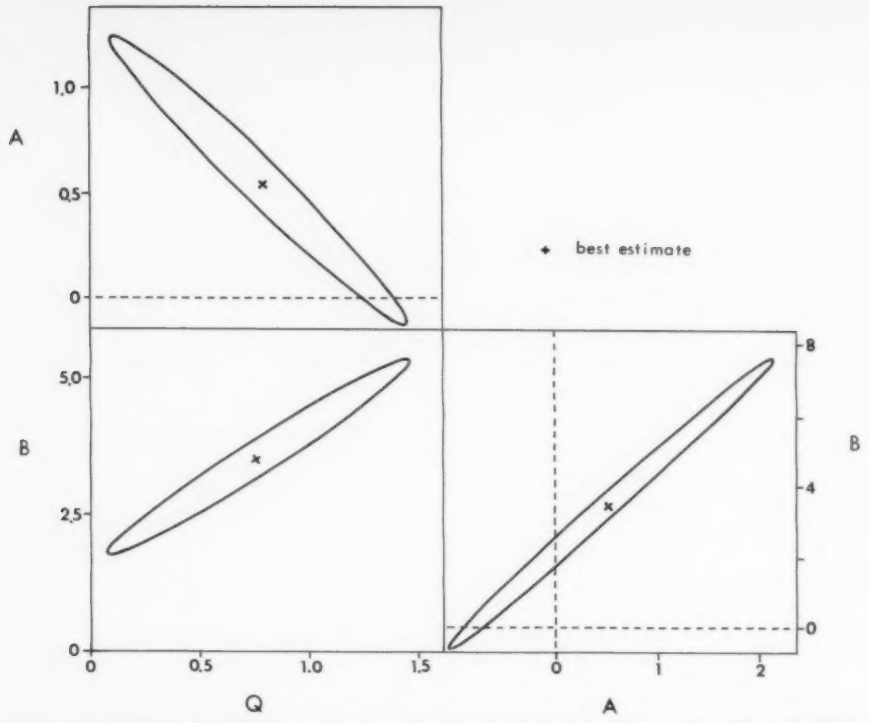

FIG. 4. Approximate joint 90% confidence regions for the a, b, and q parameters estimated by GENEST for Grand Banks yellowtail flounder. The two-dimensional sections were obtained by holding one parameter at its best estimate and varying the other two.

most realistic fits were obtained with $a = 0.6$. The estimates of maximum sustainable yield (MSY$|a = 0.6$) for Georges Bank and Southern New England (16.2 and 21.9 kt) were very close to other estimates of long-term potential catch for these stocks (16 and 23 kt; Anonymous 1989). Some convergence problems were encountered for the Scotian Shelf stock with $a < 0.6$ and for Georges Bank with $a > 0.6$. In these degenerative fits, q was unrealistically small, which means that equilibrium yields were probably overestimated. These few unrealistic parameter sets were retained in a Bayesian spirit and are balanced by the more pessimistic parameter combinations.

Equilibrium Analysis

The stock–recruitment relationships implied by the alternative parameter combinations predict different maximum recruitments and different stock sizes for obtaining the maximum (e.g. Fig. 5). Recruitment has been observed only over a narrow range of stock sizes within which the recruitment curves intersect. Additional recruitment observations within historical abundance levels will not help distinguish the alternative models. Only by observing the population respond to higher or lower stock sizes can the alternative hypotheses be distinguished. The Grand Banks stock has possibly been underexploited, unlike the other five stocks which may well have been overexploited. Without abundance contrasts in all stocks, pooled estimation of a would not help to distinguish the alternative recruitment curves.

The equilibrium yield curves (Table 1) are almost the mirror images of the recruitment curves. The low a model predicts

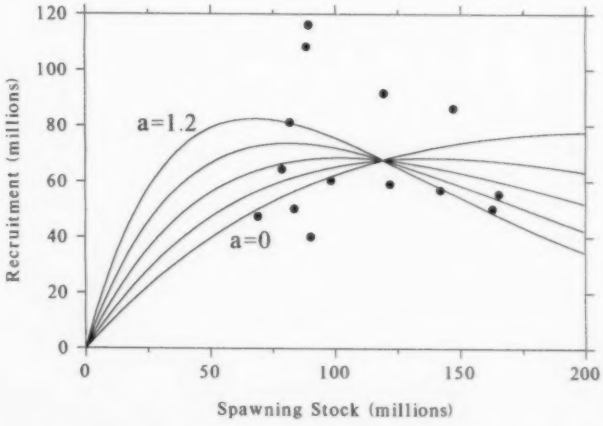

FIG. 5. Estimated stock–recruitment curves for Grand Banks yellowtail flounder. The points are cohort analysis estimates and are intended to show the range of stock sizes over which recruitment has been observed. The curves were generated by fitting the Ricker model to the cohort analysis data with the a parameter fixed. Each curve corresponds to a different Ricker a value ranging from $a = 0$ (least dome shaped) to $a = 1.2$ (most dome shaped). The recruitment curves generated for this figure are for illustration only and do not have the same b and q parameters as the set of stock–recruitment relationships obtained with the delay-difference model.

TABLE 1. Annual equilibrium yields (thousands of metric tons) of Grand Banks yellowtail flounder for different model/harvest-rate combinations. Bold type indicates the maximum equilibrium yield of each model identified by its Ricker *a* value.

Harvest rate (%)	Ricker *a* value					
	0.0	0.3	0.6	0.9	1.2	Mean
20	14.7	13.5	12.7	13.4	16.8	14.2
30	**16.6**	16.8	16.8	18.5	23.7	18.5
40	13.6	**17.0**	**18.9**	22.1	29.6	**20.2**
50	2.4	12.0	17.6	**23.5**	33.8	17.9
60	0.0	0.0	9.9	20.1	**34.2**	12.8
70	0.0	0.0	0.0	5.4	24.8	6.0
C_{max}	**16.6**	**17.0**	**18.9**	**23.5**	**34.2**	**22.0**

that the stock can sustain only a low exploitation rate and that the maximum yield is only slightly higher than the 1985–89 TAC of 15 kt. In contrast, the high *a* model predicts that high harvest rates and yields almost as high as those of the early 1970s are sustainable. Which is the optimal harvest rate and/or TAC considering that the true model is unknown Because the alternative models fit the data identically well (equal sums of squares), a mean yield curve can be simply computed as the average of the yield curves across models. The mean yield curve (Table 1) predicts a maximum yield of 20.2 kt; this is the best that could be expected given no further learning about which model is correct.

Now imagine that one is suddenly blessed with perfect information and can tell which model is correct. One does not know which model it would be, but whichever were correct, one assumes it could be managed to obtain the maximum yield; the mean of these maxima is 22 kt. The difference between the maximum expected yield with perfect information and the yield with no learning, 1.8 kt, is the expected value of perfect information (EVPI; Walters 1986). In this example the EVPI is 8.9% the catch with no learning, which may seem a marginal increase. However, given the 1988 ex-vessel price of $2.60(U.S.)/kg (Anonymous 1989), the expected increase in yellowtail flounder yield would be worth $4.7 million annually for the Grand Banks stock alone. Thus it appears from this equilibrium analysis example that it would be worth experimenting to learn which model is correct. In the following sections we investigate what the nature of such an experiment should be, how quickly one can learn, and how much additional yield is attainable.

Similarity of Subpopulations

The suitability of subpopulations as experimental replicates or controls depends, among other things, on the extent to which environmental perturbations are shared among them. This similarity can be measured from the unexplained residuals of the delay-difference equation fit to the data. The first-order autocorrelation of the residuals was significant ($p < 0.05$) for two of the subpopulations, Georges Bank and Southern New England. The residuals of the survey data appear more highly autocorrelated than the CPUE residuals. We performed cluster analysis on the correlation matrix of the residuals. The subpopulations cluster more or less geographically in that adjacent stocks are more similar than distant ones (Fig. 6). This local similarity, yet lack of coastwide shared response implies that adjacent stocks are better experimental controls for each other than for distantly removed stocks.

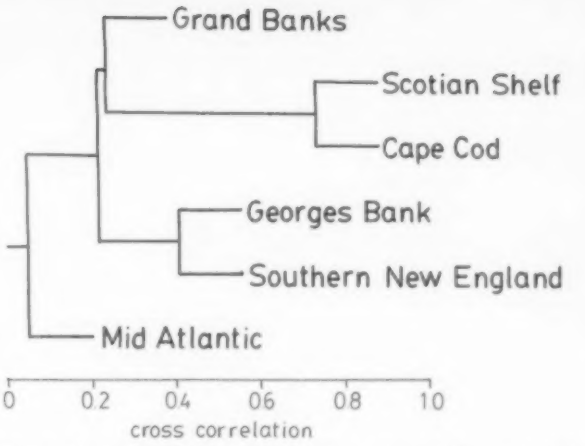

FIG. 6. Similarity of residuals (ω_t) from the delay-difference model fits to six stocks of yellowtail flounder. Cluster analysis was performed on the correlation matrix of residuals.

Stochastic Simulation Model

We constructed a stochastic simulation model to estimate the value of experimental fishing policies, applied to replicated populations. The basic model simulates the population dynamics according to the Deriso/Schnute delay-difference equation (Eq. 4), with parameter values estimated from the observed data. Superimposed on the population dynamics is a simulated manager that interprets new observations as they are collected and adjusts the harvest rates accordingly. The simulation is similar to that used by Collie and Walters (1987) except that the present model has been expanded to include up to 10 subpopulations and errors in measuring relative abundance.

Starting from 1985 abundance levels, the program simultaneously projects forward the five alternative models for each of the six subpopulations. One of the models is correct, and it is the manager's job, by setting harvest rates and gathering data, to identify the correct model. We assumed that the subpopulations share the same true Ricker *a* value because there must be some information in common for the results of experimenting on one stock to be applicable to other stocks. Otherwise it would be necessary to experiment on each stock individually, without replicates or controls. Of the three model parameters, *a* is the most likely to be shared among stocks because it is related to the intrinsic rate of increase whereas *b* and *q* depend on each stock's habitat. There is some evidence of a latitudinal gradient in yellowtail flounder productivity; for example, Grand Banks yellowtail flounder grow more slowly than the New England stocks. Ricker *a* values determined from estimated numbers at age (cohort analysis) for the three stocks with catch-at-age data (Grand Banks, Georges Bank, and Southern New England) ranged from 0.3 to 0.9 ± 0.5 (SE). There was no latitudinal gradient in estimated productivity, and the estimated *a* values were not significantly different among stocks. For the purposes of this paper we therefore accept the hypothesis of a common Ricker *a* value.

For each stock (n) and each simulated year (t) the following calculations are made. Observed relative abundance (y_o) is proportional to biomass of the correct model (B_c)

$$(5) \quad y_o = q_c B_c + \epsilon_t$$

plus a measurement error (ϵ_i) that is multivariate normally distributed with mean zero and variance–covariance matrix E as derived in the Appendix from the observed data. For each alternative model (m), predicted biomass ($\hat{B}_m$) is calculated according to the delay-difference equation (Eq. 4). The expected relative abundance of each model is $\hat{y}_m = q_m\hat{B}_m$, and the residual deviation in relative abundance is $u_m = y_o - \hat{y}_m$. The likelihood of obtaining the set of residuals (one for each stock), conditional on model m being true, is given by the multivariate normal frequency distribution:

$$(6)\quad l_m = \exp(-u_m'E^{-1}u_m/2)/[(2\pi)^n \det|E|]^{1/2}.$$

The likelihood of each model being correct (l_m) is thus related to the sum of squares and cross-products of the deviations u and variance matrix E, and a larger relative likelihood is assigned to the model with smaller residuals. The larger the cross-correlations among stocks (subdiagonal elements of E) the greater the tendency for the n residuals to have the same sign and magnitude which, in turn, speeds up the rate at which the manager distinguishes the correct model. More precisely, the likelihood function ignores or filters out the effect of large deviations that are correlated among stocks ($u'E^{-1}u$ is small for instances where such deviations occur when other components of u are held fixed), so the model discrimination proceeds as though only smaller, independent errors were present in the first place. Note that there needs to be some contrast among hypotheses in the predicted responses (apparent u values under each hypothesis), but not necessarily among stocks (components of the u vector), in order that learning can occur through assignment of different likelihoods to the hypotheses.

Bayes posterior probability $L_m(t)$ of each model being correct is updated recursively each year:

$$(7)\quad L_m(t) = l_m L_m(t-1)/D$$

where D is the probability of the data (a scaling factor). The simulation is started with equal probabilities assigned to each model and the stocks managed according to an experimental harvest policy. As the simulation proceeds, if the probability assigned to one of the models exceeds 0.9, the manager assumes this model is correct and applies the optimal harvest rate for this model the subsequent year, and for as long as $L_m > 0.9$. Should L_m fall back below 0.9, the simulated manager returns to the initial experimental harvest policy. The biomass estimates for each model ($\hat{B}_m$) are updated with the new relative abundance observation (y_o) using a Kalman filtering procedure described in the Appendix. The essence of the procedure is to average the current year's observation and the model prediction, weighted by the relative error in each type of information.

Simulation Model Results

Fifty-year simulations were run, with each of the alternative models being the correct model and with a range of experimental harvest policies. Initial experimental policies, applied whenever no model had $L_m > 0.9$, were simply the harvest rates for MSY for each model. One hundred Monte Carlo trials were performed for each model/harvest-rate combination, with each combination initiated with the same random error sequence. Learning time is defined as the number of years for the simulated manager to identify the correct model. Note that it was possible for the simulated manager to select the wrong model as correct (see $L_m > 0.9$ and act accordingly) and to reverse that wrong management decision later in the simulation.

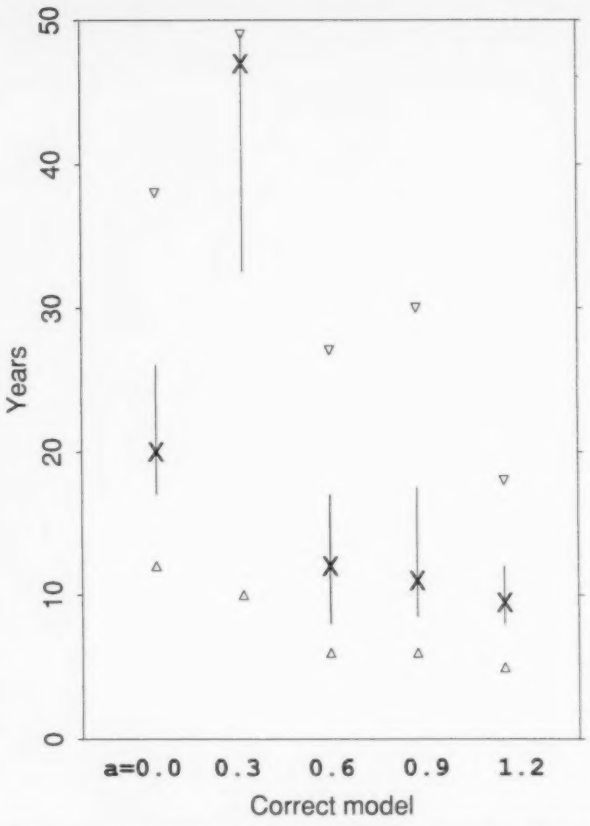

FIG. 7. Learning times measured as the number of years to identify the correct model. The initial harvest rate was 42% which is the MSY harvest rate for the $a = 0.6$ model. The modified box plots summarize the results of 100 Monte Carlo trials: the × indicates the median, the bars the quartiles, and the triangles the range in learning times.

Expected learning times depended on which was the true underlying model (Fig. 7). In this example the initial harvest rate was 42%, which is the optimal harvest rate for $a = 0.6$. Learning was fastest for the most productive model ($a = 1.2$) because the population responded quickly to any change in harvest rates. Learning was slowest for the low-productivity ($a = 0.3$) model because the populations responded more slowly and the dynamics were more easily confused with the predicted responses of neighboring models ($a = 0$, $a = 0.6$).

Overall, learning times were in the order of 10–20 yr. The main reason for this relatively rapid learning is that information was shared among subpopulations *and* the random errors among subpopulations were cross-correlated. In earlier trials, not reported here, we investigatged the effect on learning times of shared information and correlated residuals. With no shared information or uncorrelated residuals, the correct model was not identified within the 50-yr simulation. With shared information and correlated residuals, the correct model was identified in 12–15 yr, as reported in Fig. 7. Thus, spatial replication speeded learning by a factor of 3–4.

A measure of the overall value of different initial harvest policies was calculated by summing the cumulative 50-yr yields from the six subpopulations (Fig. 8). Yield was discounted 3% per annum, which means that a unit of catch in 50 yr is worth

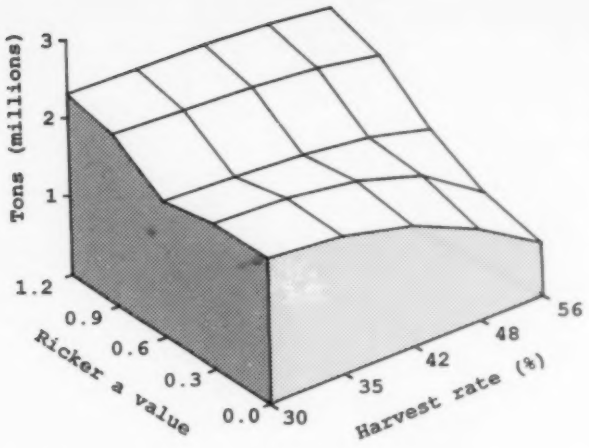

FIG. 8. Expected 50-yr yields with learning. Yield was summed over the six stocks and discounted 3% per annum. The initial harvest rates were applied until the simulated manager identified the correct (or incorrect) model. The models are identified by the Ricker *a* values.

one fifth its present value. In Fig. 8 the different correct models are represented by their Ricker *a* values. Each of the initial harvest rates is the harvest rate for MSY for each of the alternative models in ascending order. Thus, one diagonal of the surface in Fig. 8 results from applying the correct initial harvest rate to the correct model. Yields for the off-diagonal combinations result from initially applying the wrong harvest rate and attempting to learn which model is correct. Obviously, higher yields are obtained for the higher productivity models. The greatest difference in yield among models resulted from the highest initial harvest rate: when a high-productivity model was correct, high yields were obtained, but when a lower productivity model was correct, the simulated manager failed to identify the correct model and continued to overexploit the stocks.

The current position on the yield surface of Fig. 8 is difficult to locate because the correct model is unknown and because calculated harvest rates depend on uncertain estimates of population size. The most likely range for the Ricker *a* value, based on catch-at-age data, is 0.3–0.6. Yield-per-recruit analysis for the New England stocks indicated that $F_{0.1} = 0.21$ and $F_{max} = 0.54$ (Anonymous 1989). Converted to exploitation rates, these values are 0.17 and 0.38, respectively, values that lie in the lower range of the initial harvest rates used in the simulations. During the 1980s the New England stocks were severely overexploited, with fishing mortalities substantially exceeding F_{max} (Anonymous 1989). Taken together, these pieces of information suggest that the yellowtail flounder stocks are currently (1986–88) in danger of figuratively sliding off the right-hand corner of Fig. 8 into the abyss of no adaptive learning.

Additional simulation trials were conducted in which different initial harvest rates were applied to different stocks. The rationale was that learning would be accelerated by contrasting high and low harvest rates and that it would be more feasible to implement harvest rate reduction on a subset of the stocks. In this set of trials, initial harvest rates of 48% were applied to the Grand Banks, Scotian Shelf, Cape Cod, and Southern New England stocks ("controls") and 30% to the Georges Bank and Mid Atlantic stocks ("treatment"). The simulation was repeated, with each alternative model being the correct model.

TABLE 2. Mean learning times (in years) for the simulated manager to identify the correct model. Learning times are reported for all combinations of correct models and initial harvest rates. The "mixed" harvest-rate simulation applied different initial harvest rates to the yellowtail flounder stocks as follows: 48% to Grand Banks, Scotian Shelf, Cape Cod, and Southern New England; 30% to Georges Bank and Mid Atlantic.

Initial harvest rate (%)	Correct model (Ricker *a* value)				
	0.0	0.3	0.6	0.9	1.2
30	19	29	15	12	12
35	18	33	14	13	11
42	22	40	14	14	10
48	34	44	14	15	10
56	45	49*	16	18	10
Mixed	20	45	15	13	11

*No learning occurred.

As expected, the mixed harvest-rate strategy performed better than the uniform 48% initial harvest rate, especially when a low-productivity model was correct.

Surprisingly, the mixed harvest-rate policy did not result in higher yields or faster learning times than the uniformly low initial harvest rates of 30 or 35% (Table 2). With several of the stocks starting at low abundance, it appeared more informative to let them all rebuild than to create harvest-rate contrasts. There are two reasons for this result: (1) even ignoring the value of information, the optimum initial harvest rate (to maximize 50-yr yield) is lower than the optimum harvest rate for long-term equilibrium, since the stocks were overexploited to begin with; and (2) the alternative models to be discriminated differed only in the *a* parameter, which is best estimated by allowing the most rapid possible population growth. No information about *a* is provided by contrasting stocks against one another.

One might intuitively expect that mixed harvest rates would constitute a better experimental design, by creating contrasting abundance patterns over the set of subpopulations. This would indeed be the case if we had assumed that all stocks share the same (and unknown) basic production relationship. With all parameters shared, the most informative experiment would be to gather observations (across stocks and over time) over a broad range of values for the independent variable (stock size) of the stock–recruitment relationship. However, our method of structuring alternative hypotheses (based on historical data) did not assume the existence of such a shared relationship and admitted uncertainty only concerning shared values of productivity (Ricker *a* value) at low stock sizes. Creation of contrasting behavior among stocks is not the best strategy when only a particular key feature of all stocks is to be estimated; in this case, it is best to treat them all the same and hence obtain better replication in measurement of that feature.

In the absence of learning, the 42% initial harvest rate resulted in the highest yield averaged across alternative models (1.825 million metric tons (Mt)). With learning, the highest average yield (1.921 Mt) was obtained with a lower initial harvest rate of 35%. One value of the lower harvest rate is that the stocks rebuilt more quickly, making it easier to distinguish the correct model. With perfect information, one could obtain the maximal yield from each model and the average of these yields across models was 1.974 Mt. The expected value of perfect information, calculated from the simulation model results for all stocks combined, was thus 1.974 − 1.825 = 0.149 Mt, or 8.2% of the expected yield with no learning. The expected

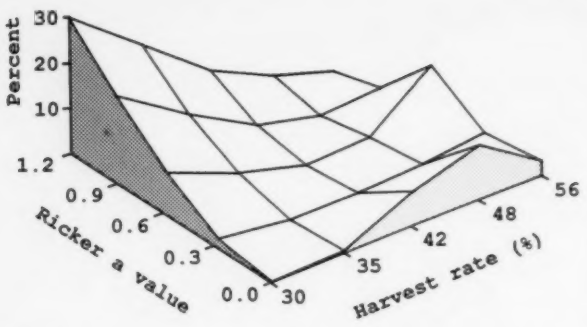

FIG. 9. Increase in yield from learning over nonlearning simulations.

increase in yield with learning was 1.921 − 1.825 = 0.096 Mt, or 5.3%. In this example the EVPI was relatively small but the simulated adaptive manager was able to obtain most of the potential increase in yield.

The value of learning is emphasized by plotting the difference in yields in simuluations with and without learning (Fig. 9). Obviously there is no value of learning if one happens to initially apply the optimal harvest rate for each model, and this accounts for the valley of zeros in Fig. 9. However, if an incorrect initial harvest rate is applied, there is an increasing value of learning and correcting the mistake. In the extreme case of applying the lowest harvest rate to the most productive model, the increase in yield from learning was 30%. We expected the valley in Fig. 9 to be symmetrically *U*-shaped such that the value of learning would increase smoothly in either direction from the zero diagonal. Instead there was a canyon leading to the right-hand corner. These low values resulted from applying too high an initial harvest rate to a low-productivity model, in which case the stocks never recovered enough for the correct model to be distinguished. As mentioned above, it is likely that the stocks currently (as of 1988) lie in this Canyon of No Learning.

Discussion

We have developed and demonstrated a methodology for evaluating adaptive management policies applied to spatially replicated groundfish populations. The method is applicable to subpopulations which have an uncertain demographic parameter in common and a shared response to environmental factors. The simulated adaptive manager was successfully able to distinguish among alternative models of the population dynamics of yellowtail flounder in 10–20 yr. The success of this method depended on several assumptions about the population models; the validity of these assumptions is discussed in the following paragraphs.

A critical assumption is that the subpopulations are discrete. While this appears to be true for adult yellowtail flounder, there is a possibility of exchange among the New England stocks during the 3- to 4-mo pelagic phase (Lux and Livingstone 1982). While a larva could conceivably travel from Georges Bank to the Mid Atlantic grounds in 4 mo, the summer gyre on Georges Bank (Butman et al. 1987) may retain larvae. It is likely that yellowtail flounder larvae, like other marine fish larvae, navigate to some extent by vertical migration. Some form of retention and/or juvenile migration would appear necessary to maintain the large stocks of yellowtail flounder. Any significant exchange among stocks would compromise an experimental strategy that applied differential harvest rates to adjacent stocks. The solution is to either apply a common exploitation rate to all stocks (as in the simulations) or to conduct harvest-rate experiments on widely separated stocks (e.g. Southern New England and Grand Banks).

While it is hoped that the subpopulations do not actually share fish, it is assumed that they share at least some demographic parameters. This is essential if information gained from one stock is to be applied to other stocks. Of the three free parameters of the delay-difference model, the Ricker *a* parameter is the most likely to be similar among stocks, and it also uniquely determines the MSY harvest rate. At present, the standard error of estimated productivity (*a*) of individual yellowtail flounder stocks exceeds the differences in estimated productivity among stocks. A rigorous method for testing the common *a* assumption is to fit a pooled regression model to all stocks simultaneously with either a shared *a* or individual *a* parameters. This procedure was used to support the common *a* assumption for Fraser River sockeye salmon, *Oncorhynchus nerka* (Collie et al. 1990). While a pooled regression is relatively straightforward for a linearized production model (e.g. Polovina 1989), the estimation would be numerically much more difficult for a nonlinear model such as the delay-difference equation.

A third assumption, necessary to make the problem tractable, was that all the parameter uncertainty was represented by the major axis of the confidence regions (e.g. Fig. 4) and that once the *a* value was chosen, *b* and *q* were fixed. In fact there was some uncertainty in the *b*–*q* plane independent of *a*, which means that the yields predicted for a given value have an additional degree(s) of uncertainty. While the Ricker *a* parameter determines the harvest rate for MSY, *q* (and *b*) determines the yield obtained at that harvest rate. If the *a* and *q* (and *b*) estimates are positively correlated, the different models (*a* values) may have similar MSYs even though the yields are obtained at different exploitation rates. This appeared to be the case for the Cape Cod and Southern New England stocks and it tends to make the yield surface of Fig. 8 more level than it might be otherwise. We attribute this result more to lack of model fit for these stocks than to stability of the MSY estimate.

A final assumption was in identifying alternative population models by discrete parameter sets rather than continuous distributions. The discrete model assumption makes the calculations involved in adaptive learning more tractable and permits the inclusion of alternative models with different functional forms. However, when we say, for example, that the correct model is $a = 0.6$, we really mean that the correct value of *a* lies somewhere in the range 0.45–0.75. This implies an additional element of parameter uncertainty that would tend to lengthen the learning times in Fig. 7.

The set of alternative models considered for yellowtail flounder was not meant to be exclusive. Indeed the philosophy of adaptive management is to entertain all reasonable models. The alternative parameterizations of the delay-difference model were useful for characterizing the uncertainty in yellowtail flounder productivity. Instead of trying to distinguish among alternative stock–recruitment curves, given the scatter in a typical plot (e.g. Fig. 5), one might ask whether there is a stock–recruitment relationship at all. Whether or not one believes in stock–recruitment curves, some form of stock–production relationship is necessary to satisfy the conservation mandate of fisheries management. The purpose of the stock–recruitment curves is to predict *mean* recruitment at each stock size, not to

predict actual recruitment in a given year. If recruitment could actually be predicted, adaptive management would be unnecessary.

Some of the recruitment variability, not explained by the delay-difference model, may be explained by environmental factors. For example, Sissenwine (1977) simulated the yield of Southern New England yellowtail flounder with a model that included variable fishing effort and the effects of temperature variations superimposed on underlying stock–recruitment functions. Myers and Drinkwater (1989) found a negative relationship between recruitment of 17 groundfish stocks (including yellowtail flounder) and an index of entrainment by Gulf Stream warm core rings. It is possible that inclusion of these environmental factors might refine the estimated mean stock–recruitment relationships, and there is provision in GENEST to include such factors as recruitment multipliers. The value of using environmental correlates to predict recruitment is beyond the scope of this paper; the topic was recently addressed by Walters (1989).

Groundfish stocks are commonly managed by applying a target exploitation rate to the current biomass estimate to obtain a TAC. We attempted to simulate this management policy by setting the TAC based on biomass estimated by the model with the highest probability, not the correct (but unknown to the manager) biomass. To ensure that the TAC did not exceed the true biomass, when true biomass was considerably lower than estimated biomass, catch was constrained by the maximum fishing effort level observed for each stock. Even with the maximum effort constraint the simulated manager was unable to protect the stocks from irreversible overfishing. This occurred because of the 1-yr lag between gaining new relative abundance data and acting on it by setting a new TAC. Managers of most groundfish stocks must always react to, rather than anticipate, changes in abundance. The inability of the simulated manager to prevent overfishing may thus be realistic. We revised the simulated management policy by simply applying a target exploitation rate to the biomass of the correct model.

The policies actually used to manage yellowtail flounder differ among stocks. Grand Banks yellowtail flounder have been under TAC regulation since 1973 (Brodie 1986). Scotian Shelf yellowtail flounder are under quota management as part of a groundfish complex (Neilson and Perley 1986). In the United States, yellowtail flounder have been managed under a Fisheries Management Plan since 1977. Two management units were established: Georges Bank (east of 69°W) and a second unit (west of 69°W) encompassing the Cape Cod, Southern New England and Mid Atlantic stocks (Clark et al. 1984). Management measures implemented in New England at one time or another include quotas, minimum codend mesh sizes, trip limits, a minimum possession size, and data reporting requirements. An adaptive management policy could be implemented under existing regulations as a set of TACs and would probably require U.S.–Canada coordination.

The main conclusion of this study is that it is worth adjusting harvest rates to account for the value of learning more about the population dynamics of groundfish. In the yellowtail flounder example, it was worthwhile to reduce the harvest rates below the apparently optimal level with no learning. This result may seem to simply support harvesting at the $F_{0.1}$ rate instead of F_{max}, but the result was contingent on the current low abundance of most yellowtail flounder stocks. Had most or all of the stocks been more abundant, the optimal adaptive policy might have been to harvest above the level for apparent MSY. Conclusions about the value of adaptive management can only be drawn on a case-by-case basis. The purpose of the simulation model is not to predict the future but to determine whether improved information is worth its cost.

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Appendix

This appendix derives the equations used in the Monte Carlo simulations. Vector notation is used, with each vector element representing one of the six subpopulations. For each random trial with the same true model and for each year of the simulation (t), the vector of true biomasses (B_t) is calculated according to the delay-difference equation (Eq. 4). A discrete catch (C_t) is calculated by applying a harvest rate (h_t) to the correct biomass such that $P_t = (1 - h_t)B_t$. Observed relative abundance (y_o) is proportional to correct biomass with an additive error:

$$\text{(A1)} \quad y_o = QB_t + \epsilon_t.$$

The matrix Q is a diagonal matrix and contains the catchability coefficients (q_n) of the correct model for each stock. The vector of errors ϵ_t has variance–covariance matrix E as derived below.

For each alternative model (m) and for each stock, biomass in year t is predicted from data up to and including year $t-1$:

$$\text{(A2)} \quad \hat{B}_{t|t-1} = s(1+\rho)\hat{P}_{t-1} - \rho s(\hat{P}_{t-1}/\hat{B}_{t-1})(s\hat{P}_{t-2} + v\hat{R}_{t-1}) + \hat{R}_t.$$

Here, $\hat{R}_t$ is the predicted recruitment rate from the stock–recruitment equation for model m. The expected relative abundance of each model is $\hat{y}_m = Q_m\hat{B}_{m,t|t-1}$, and the vector of residuals for each model is $u_m = y_o - \hat{y}_m$. The likelihood of each model being the correct model is

$$\text{(A3)} \quad l_m = \exp(-u_m'E^{-1}u_m/2)/[(2\pi)^n \det|E|]^{1/2}.$$

Finally, the biomass estimates for each model are updated to account for the relative abundance observation in year t:

$$\text{(A4)} \quad \hat{B}_{t|t} = \hat{B}_{t|t-1} + \overline{K}u_t.$$

Equations A2–A4 are calculated for each alternative model before proceeding to the next year.

The Kalman gain matrix ($\overline{K}$) weights the relative abundance residuals relative to the delay-difference model predictions. A small gain places a low weight on the relative abundance index, while a large gain places a low weight on the model predictions. A Kalman gain matrix is defined for each model by the following recursive equations:

$$\text{(A5)} \quad V_{t|t-1} = A'V_{t-1|t-1}A + \text{Var}(R)$$

where $V_{t|t-1}$ is the variance–covariance matrix of biomass as predicted by the delay-difference equation and $V_{t-1|t-1}$ is the covariance matrix of the biomass estimate for $t-1$, updated by all information available up to and including time $t-1$. Matrix A is diagonal, with each element equal to $s(\alpha/\overline{w} + \rho)$ for each stock (from Eq. 1). Var(R) is the variance–covariance of recruitment calculated from cohort analysis estimates of recruitment converted to weight units. Note that Var(R) represents a process error that the stimulated manager must admit is present when calculating covariance matrix V, even though we did not include such errors in the GENEST model used to estimate the parameters for the simulation. The Kalman matrix is

$$\text{(A6)} \quad K_t = V_{t|t-1}Q_m(Q_m'V_{t|t-1}Q_m + \Omega)^{-1}$$

where Ω is the variance–covariance matrix of observation errors calculated from the residuals of the delay-difference model fit. Finally, V is updated to reflect the observation of relative abundance in year t:

$$\text{(A7)} \quad V_{t|t} = (I - K_tQ_m)V_{t|t-1}$$

where I is the n^*n identity matrix. If A is assumed to be constant (w does not change), the set of equations A5–A7 converges within a few iterations. This is computationally convenient because equilibrium values $\overline{V}$ and $\overline{K}$ can be calculated once for each model outside the main simulation loop (A1–A4; $\overline{V}$ is the value of $V_{t|t}$ after enough time that $V_{t|t} = V_{t+1|t+1}$).

The variance–covariance matrix of simulated errors is $E_m = \Omega + Q'_m\overline{V}_mQ_m$. Total error is thus a sum of observation error (Ω) plus state estimation errors ($\overline{V}$) scaled by the catchability coefficients to be in relative abundance units. Strictly speaking, there is a different Ω for each model, but because the model fits were so similar, we used the Ω for $a = 0.6$ to generate observation errors for all the models. The first simulated year was 1986. Because the maximum age at recruitment was 5 for Grand Banks, 5 yr of catch and relative abundance data (1980–85) were required to initialize the simulation. Although the different models had different relative abundance estimates for 1980–85, the estimates were so similar that predicted relative abundance for the $a = 0.6$ model was used to initialize all the alternative models.

Effects of Suspended Sediment on Growth, Respiration, and Excretion of the Soft-Shell Clam (*Mya arenaria*)

Jonathan Grant and Bruce Thorpe

Department of Oceanography, Dalhousie University, Halifax, N.S. B3H 4J1, Canada

Grant, J., and B. Thorpe. 1991. Effects of suspended sediment on growth, respiration, and excretion of the soft-shell clam (*Mya arenaria*). Can. J. Fish. Aquat. Sci. 48: 1285–1292.

In long-term exposures for 35 d, soft-shell clams (*Mya arenaria*) were maintained in a flow-through suspension of intertidal sediment (100–200 mg·L^{-1}) and in a control treatment of ambient sand-filtered seawater, each with microalgae as a food source. Weekly measurements demonstrated a significant decrease in oxygen consumption and increase in ammonia excretion through time in the turbidity treatment compared with controls. Atomic O:N ratios fell to 14 for exposed clams and remained at 27 for controls, suggesting that exposed clams were utilizing body reserves to meet nutritional needs. Dry tissue weight showed no difference between initial and final values in the presence of suspended sediments, but control animals grew by 43%. In short-term exposures to 0–2000 mg suspended sediments ·L^{-1}, oxygen consumption declined sharply with sediment concentration, suggesting that inhibition of respiration occurred at low values of turbidity. In further short-term observations, the threshold for the production of pseudofeces was 100–119 mg·L^{-1}. These results suggest that *Mya arenaria* copes with increased turbidity by reducing its ventilation rate, a response which provides tolerance to intermittent turbidity, but causes starvation during long-term exposure.

Soumises à des expositions de longue durée (35 jours), des myes (*Mya arenaria*) ont été maintenues dans une suspension à écoulement continu de sédiment intertidal (100–200 mg·L^{-1}) et dans des conditions témoins d'eau de mer ambiante filtrée dans du sable, avec dans les deux cas des microalgues comme source de nourriture. Des mesures hebdomadaires ont montré une diminution significative de la consommation d'oxygène et une augmentation de l'excrétion d'ammoniac en fonction du temps dans la suspension par rapport au bain témoin. Les rapports O:N au niveau atomique sont tombés à 14 pour les myes exposées et sont restés à 27 pour les coquillages témoins, ce qui semble indiquer que les myes exposées utilisaient leurs réserves corporelles pour répondre à leurs besoins en nourriture. On n'a observé aucune différence dans le poids sec de tissu entre les valeurs initiales et finales en présence de sédiments en suspension, mais les animaux témoins ont montré une croissance de 43 %. Dans des expositions de courte durée à des sédiments en suspension dans des concentrations de 0–2000 mg·L^{-1}, la consommation d'oxygène a baissé fortement en fonction de la concentration de sédiments, ce qui semble indiquer une inhibition de la respiration aux faibles valeurs de turbidité. Dans d'autres observations de courte durée, le seuil de production de pseudofèces était de 100–119 mg·L^{-1}. Ces résultats semblent indiquer que *Mya arenaria* s'adapte à une augmentation de la turbidité en réduisant son taux de ventilation, réaction qui assure une tolérance à une turbidité intermittente, mais cause l'inanition en cas d'exposition de longue durée.

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High concentrations of suspended solids (turbidity) in marine environments arise from a variety of natural and anthropogenic sources (Moore 1978; Appleby and Scarratt 1989). Plankton blooms, bioturbation, soil erosion, flocculation, and wave/current resuspension of sediments all contribute to natural suspended particle loads. In addition, ocean dumping, agriculture, boating, dredging, and marine construction can increase turbidity of the water column. Suspended solids have a variety of effects on aquatic organisms including reduced light and primary production (Cloern 1987), alteration of zooplankton and fish feeding (Hart 1988; Grecay 1989), mobilization of pollutants (Cooper 1988), and smothering of benthic communities. In particular, suspension feeding fauna such as lamellibranch bivalves are stressed by excessive turbidity (Levinton and Bambach 1969; Peterson 1985). Both field and laboratory studies have demonstrated reduced feeding activity and/or growth of bivalves in the presence of high suspended loads caused by either sediment transport or bioturbation (Bricelj and Malouf 1984; Bricelj et al. 1984; Griffiths and Griffiths 1987; Grant et al. 1990).

Where bivalves are cultured or harvested commercially, changes in turbidity can have a severe impact on fishery production. In eastern Canada, one such impact may arise from the construction and operation of a tidal power station in the Bay of Fundy (Gordon 1984). The >10 m tidal range in the upper bay creates extensive tidal flats which support a fishery for the soft-shell clam (*Mya arenaria*). The Annapolis Estuary, near Digby, comprises up to 68% of the Nova Scotia clam fishery (Angus et al. 1985). In 1960, a causeway was constructed across the Annapolis River, and a turbine and pilot tidal power station were completed in 1984 (Redden et al. 1984; Daborn and Dadswell 1988). Over the last several years, local fishermen have expressed concern that some aspect of this tidal power plant has altered the estuarine environment in a way deleterious to clam stocks (Prouse et al. 1988).

Tidal power may produce a variety of habitat changes, including alteration of current speeds and patterns (Daborn 1983) and enhanced settling and near-bottom concentrations of suspended sediments (Amos 1984). Modifications in current

regime due to tidal power development will potentially expose suspension feeders such as *Mya arenaria* to elevated concentrations of suspended particles. Moreover, excavation and construction phases of the power station will add to suspended sediment loads.

Mya arenaria fisheries are minimal in the highly turbid uppermost Bay of Fundy (Cobequid Bay). Newcombe (1935) suggested that reduced clam growth in some Fundy intertidal areas was due to a mobile mud layer which interfered with feeding. Although most of the above environmental impacts from tidal power development are speculative, it is likely that they will involve changes in suspended loads near the sediment–water interface. To further understand turbidity impacts on the benthos from the pilot plant at Annapolis Royal, and any future large-scale tidal power projects, we have conducted laboratory studies on the physiology and growth of *Mya arenaria* in the presence of suspended sediments.

Bivalves use various mechanisms to deal with high concentrations of suspended particles, many involving reduction in net pumping rates (Foster-Smith 1976). We hypothesized that reduced pumping in *Mya arenaria* would lead to decreased food intake and growth rate. Because direct measurement of feeding rate by particle removal is difficult in turbid suspensions, we used aerobic respiration rate as a measure of the clam's ability to pump water of varying turbidity. In suspension feeding bivalves, oxygen consumption is highly correlated with filtration rate during feeding (Griffiths and Griffiths 1987). The causal mechanism of the positive relationship between rates of respiration and ventilation (pumped water transport) is unclear (Jørgensen et al. 1986); does increased ventilation activity require higher oxygen uptake, or does increased oxygen consumption necessitate higher ventilation? Previous studies have demonstrated the applicability of this relationship to *Mya arenaria*. After tidal exposure to air, *Mya* relieves its oxygen debt by increased ventilation (Van Dam 1935). Increased heart rate is also associated with greater ventilation (Lowe and Trueman 1972). In both cases, respiration and ventilation are coupled to the extent that we can use oxygen consumption as an indicator of ventilation rate in this species.

In the present study, we carried out long-term exposure experiments using natural silt-clay to determine the effects of turbidity on behaviour, growth, and respiration of *Mya arenaria*. Simultaneous measurements of ammonia excretion were made to calculate atomic oxygen to nitrogen (O:N) ratio, an index of physiological condition which is highly sensitive to nutritional state (Bayne et al. 1985). Turbidity-stressed clams were expected to show reduced respiration, but increased ammonia excretion from catabolism of tissue protein as an energy source. Short-term exposure experiments were also done to measure the threshold at which suspended sediment would affect respiration and initiate the production of pseudofeces.

Materials and Methods

Mya arenaria were dug in October 1988 (long-term experiments) and June 1989 (short-term experiments) from a mudflat at Five Islands, Minas Basin, Nova Scotia. Maximum shell length (L, millimetres) of clams measured with digital calipers ranged from 42 to 69 mm (mean = 58.4 ± 4.9 (SD)). A subsample of 20 animals from each collection was used to calculate a length–weight regression, with tissue weight (W, grams) determined after drying at 70°C for 30 h (October; $r = 0.98$, $p < 0.001$):

$$(1)\quad W = 2.32 \times 10^{-5} L^{2.68}.$$

Due to the smaller range of size classes selected for June experiments, a linear relationship was found ($r = 0.84$, $p < 0.001$):

$$(2)\quad W = 4.86 \times 10^{-2} L - 0.58.$$

Clams collected in June had a much higher tissue weight for a given length. This difference was expected because *Mya* is reproductively ripe in the summer, but largely spent by the fall (Battle 1932). These length–weight regressions were used to estimate the initial weight of clams used in long-term exposures and to adjust respiration in short-term exposures to a standard size clam.

To allow for variance in respiration rate due to clam size, observed rates (R_{obs}) for clams of known weight (W_{obs}) were standardized (R_{std}) to a clam of 2 g dry weight (W_{std}):

$$(3)\quad R_{std} = (W_{std}/W_{obs})^{b} R_{obs}.$$

Emerson et al. (1988) determined an allometric exponent for *Mya arenaria* (Nova Scotia) of $b = 0.558$.

Prior to the start of long-term exposure experiments (5 wk duration), clams were acclimated at Dalhousie for several weeks at ambient temperature (7°C) in a flow-through tank and fed dry cereal (Emerson 1990). Clams were then moved to the Bedford Institute of Oceanography where the exposure system was set up and held for 2 wk in flowing sand-filtered seawater. Sediment used for turbidity exposure was intertidal mud collected from Burntcoat Head, Nova Scotia, a Minas Basin site with *Mya* populations (Bousfield and Leim 1959; pers. obs.), and convenient for transporting large quantities of mud to the laboratory. Sediment was kept cool and dark in buckets. Coulter counter analysis of suspended sediment following settlement of heavier sand fractions indicated that 35% of particles were clays (1–4 μm size range), 42% silt (5–50 μm), and 23% very fine sand (64–102 μm). Sediment organic content determined by ashing was 1.8% by weight.

In the turbidity exposure system, outflow from an aerated sand-filtered seawater head tank (6–10°C, 30–32‰) was divided into two large holding tanks: a control treatment and a sediment exposure treatment. From the head tank the latter supply entered a stirred mixing tank to which 1 L of sediment slurry was added twice daily. The mixing tank overflowed into a baffled settling bucket to retain sand fractions and then into the exposure tank. The amount of added sediment was regulated to obtain exposure concentrations of 100–200 mg sediment·L^{-1}. Flow through the system was 500–700 mL·min^{-1}, and temperature was regulated at 10°C. Fifteen clams were measured in length and placed in both control and turbidity tanks. The mean shell length of control and exposure clams was 45.9 ± 3.0 (SD) and 46.8 ± 2.1 (SD) mm, respectively. Food consisted of ~1.8 g of spray-dried *Tetraselmis* sp. (Cell Systems Ltd., Cambridge, U.K.) mixed into 2 L of seawater and dripped daily into holding tanks.

At the start of the experiment, six randomly selected clams were placed individually into magnetically stirred respirometers (0.31 L of filtered seawater; 8°C) to determine oxygen consumption and ammonia excretion. Following a 30-min acclimation period, initial oxygen concentration was determined with a YSI oxygen electrode. After a 45-min sealed incubation, tops were removed and a final oxygen concentration

determined immediately with the oxygen electrode. Incubation time was chosen to ensure a reduction of oxygen <20%. Control chambers without clams were also incubated. Initial and final water samples were taken for ammonia analysis using the phenol–hypochlorite method as in Bayne et al. (1985). Respiration and excretion experiments were conducted weekly for both control ($n = 6$) and exposure ($n = 6$) treatments. At the end of the experiment, shell length and dry tissue weight of clams were measured. Initial dry tissue weight was predicted from Eq. 1. Due to the very small range of shell length used, no attempt was made to standardize respiration to weight.

Prior to short-term exposure experiments, clams were held in sand-filtered seawater at 12°C for 7 d. A small quantity of mud was collected from the same flat as the clams (Five Islands), frozen, dried at 70°C for 48 h, and ground with mortar and pestle. After settlement of the larger sand fractions, Coulter counter analysis indicated that suspensions consisted of 16% clay (1–4 μm), 59% silt (5–50 μm), and 25% very fine sand (64–102 μm). Sediment organic content determined by ashing was 2.4%. Larger respirometers (0.9 L) were used to ensure exposure time >1 h without excessive oxygen consumption. The increased water surface of these chambers required collection of a gas-tight syringe sample for oxygen measurement rather than using the YSI electrode. Individual clams (mean 48.4 mm ± 3.8 SD) were placed into respirometers (10–12°C water bath) for 30 min of acclimation after which an initial water sample was taken for oxygen content using a Radiometer blood gas analyzer. Weighed sediment mixed in seawater was then added to the respirometer resulting in initial concentrations of suspended sediment in each stirred chamber of 0, 1, 5, 10, 20, 50, 100, 200, 500, 1000, or 2000 mg·L^{-1}. After a 90-min incubation period, water samples for oxygen determination were withdrawn by syringe. Several controls with sediment suspensions and no clams were included, but control consumption values were low regardless of sediment concentration. After clams wre removed from respirometers, the water was poured off without resuspending the settled material and vacuum filtered through a GFF filter to determine final sediment concentration.

For determination of pseudofecal threshold, individual clams were placed into 4-L buckets of seawater (7–8°C). After 30 min, Five Islands sediment was added as above in weighed amounts to achieve concentrations of 0, 4, 8, 12, 16, 20, 24, 48, 72, 96, 120, 144, 288, 576, 864, 1152, 1728, 2304, and 2880 mg·L^{-1} and kept in suspension by magnetic stirrers. After 2 h of exposure, clams were placed into white buckets of filtered seawater to check for pseudofeces. Three clams were observed for each concentration.

Results

Long-Term Exposure

In the initial stages of the experiment, *Mya arenaria* in both control and turbidity treatments displayed widely open valves with siphons and mantle edge extended, representative of clams in an unstressed state. Both groups reacted to mild physical stimulus by reducing gape and retracting siphon and mantle. These reactions are typical in that *Mya* is highly sensitive to mechanical stimuli (Jørgensen and Riisgård 1988). Control clams exhibited no change in this behaviour throughout the experiment. In contrast, the exposed group showed reduced valve gape and partial retraction of siphon and mantle after 7 d.

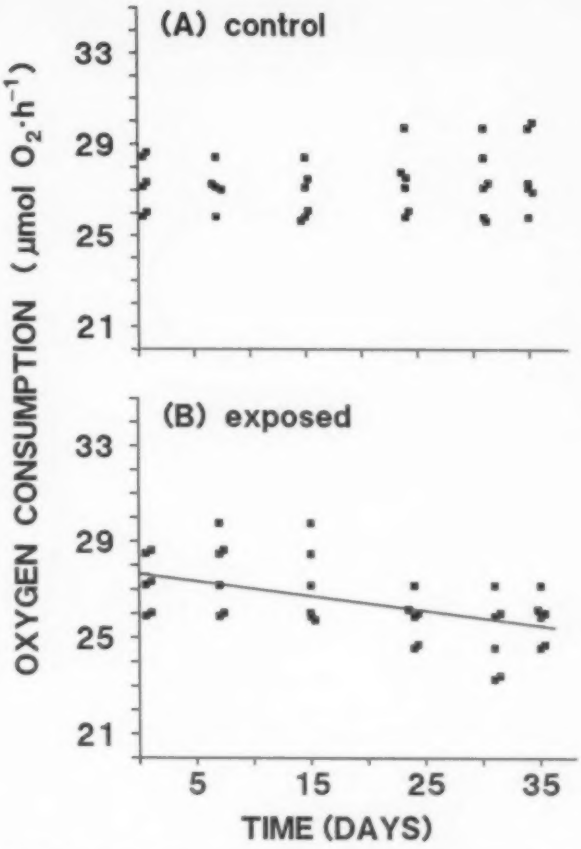

FIG. 1. Rate of oxygen consumption of individual *Mya arenaria* during 35 d of exposure to 100–200 mg suspended sediments·L^{-1}. (A) Control treatment receiving filtered seawater with a microalgal supplement. (B) Clams exposed to suspended sediments with a microalgal supplement. The regression equation for exposed clams is O_2 consumption = −0.06 (days) + 27.55 ($r = 0.50$, $p < 0.01$, $n = 36$).

By day 15, exposed clams had regained a normal appearance, but were less responsive to mechanical stimulus. This condition continued until day 30, when excessive protrusion of the siphon was observed, accompanied by even less sensitivity to handling. Appearance of the siphons differed substantially; those of the control animals remained normal in texture and colour, but siphons of exposed clams were darker in colour and covered with numerous protuberances.

Relatively small changes in oxygen consumption occurred during the experiment, but the temporal course of aerobic respiration was distinct between treatments. Linear regression indicated that the control group showed no significant change in oxygen consumption through time ($p = 0.25$; Fig. 1A) whereas the exposed group displayed a significant decline ($p < 0.01$; Fig. 1B). Analysis of covariance confirmed that exposure to turbidity had a significant factor effect ($p < 0.002$) on respiration rates through time. Mean oxygen consumption rates in the exposure treatment declined from 27.2 ± 1.2 (SD) μmol O_2·ind.$^{-1}$·h^{-1} to a final value of 25.6 ± 0.1 (SD) μmol O_2·ind.$^{-1}$·h^{-1}, a reduction of ~6% over the 35-d period. The exposure data suggest a nonlinear trend in respiration with an

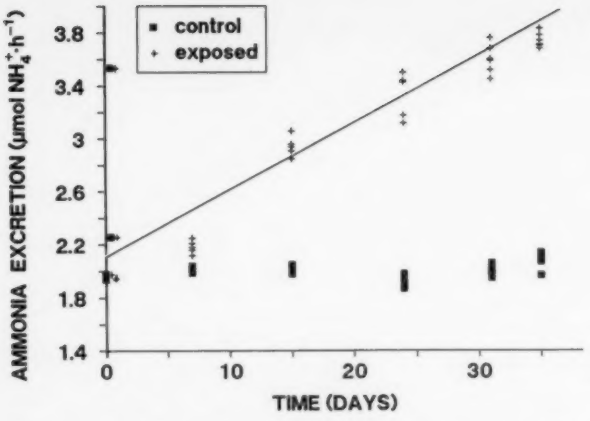

FIG. 2. Rate of ammonia excretion by individual *Mya arenaria* during 35-d experiments with suspended sediments. Control clams received only filtered seawater and microalgal food; exposed clams refers to turbidity treatment. The regression line for exposed clams is NH_4^+ excretion = 0.05 (days) + 2.15 ($r = 0.90$, $p < 0.001$, $n = 36$).

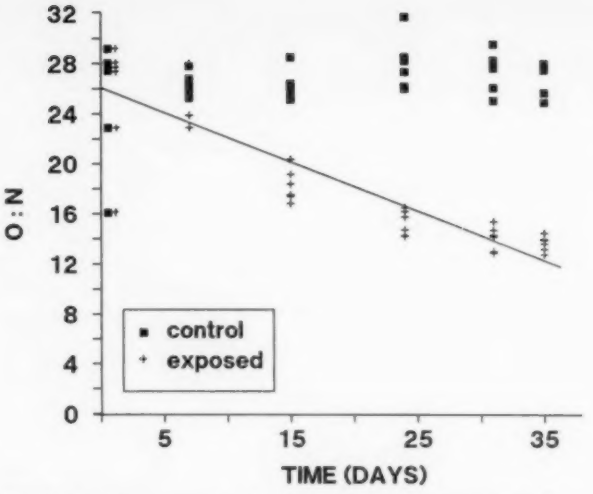

FIG. 3. Atomic oxygen to nitrogen (O:N) ratios determined from measurements of oxygen consumption and ammonia excretion in individual *Mya arenaria* during 35-d experiments with suspended sediments. Controls received filtered seawater and microalgae; exposed clams refers to turbidity treatment. The regression line for exposed clams is O:N = −0.37 (days) + 25.50 ($r = 0.88$, $p < 0.001$, $n = 36$).

apparent decline in respiration rate midway through the experiment. However, we refrain from statistical testing between these periods because they were not set up as a priori treatments.

Differences in ammonia excretion between control and exposed clams (Fig. 2) were more striking than oxygen consumption trends. Although there was no significant relationship between NH_4^+ excretion and time in the control ($p = 0.201$), ammonia excretion increased through time in exposed animals ($p < 0.001$). After 2 wk, excretion in the turbidity treatment diverged widely from the control value. Mean excretion rate of exposed clams increased from 2.0 ± 0.1 (SD) to 3.7 ± 0.1 (SD) μmol $NH_4^+ \cdot$ind.$^{-1} \cdot h^{-1}$, an overall change of 86%.

As expected from the above results, the ratio of oxygen consumption to ammonia excretion (O:N in atomic equivalents) showed no trend over time for control clams ($p = 0.135$; Fig. 3), but declined in exposed clams throughout the experiment ($p < 0.001$; Fig. 3). The mean O:N ratio for exposed animals decreased from 27 to 14 during this period.

For control clams, mean dry tissue weight was 43% higher after the experiment than mean initial weight (two-tailed *t*-test, $p < 0.001$; Fig. 4). Mean weight of exposed clams before and after the experiment was similar (two-tailed *t*-test, $p > 0.5$). Although 5 of 15 clams in the exposure treatment gained weight, these increases were <18% of initial weight. These results demonstrate that the ration of microalgal food presented was sufficient for clam growth in the control treatment, but that turbidity inhibited feeding and growth in the suspended sediment treatment.

Short-Term Exposure

In short-term experiments, we expected reduction of paticle concentration due to passive settling and/or clearance of material by clams. Initial (*I*) and final (*F*) sediment concentrations (milligrams per litre) in the respirometers showed a log–log relationship typical of passive settling phenomena, with rapid initial settlement of larger particles (sand) and a background

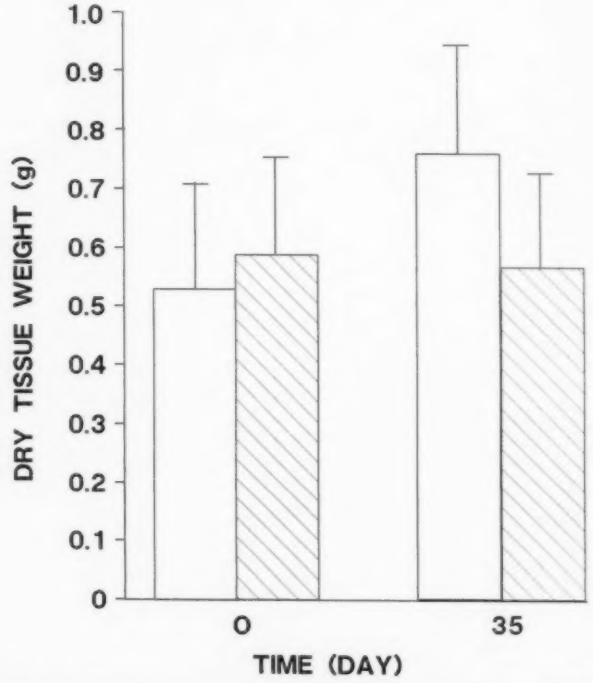

FIG. 4. Comparison of mean dry tissue weight (+SD, $n = 15$) of control clams (open bars) and clams exposed to turbidity (100–200 mg·L^{-1}, cross-hatch bars) at the start (day 0) and end (day 35) of the experiment.

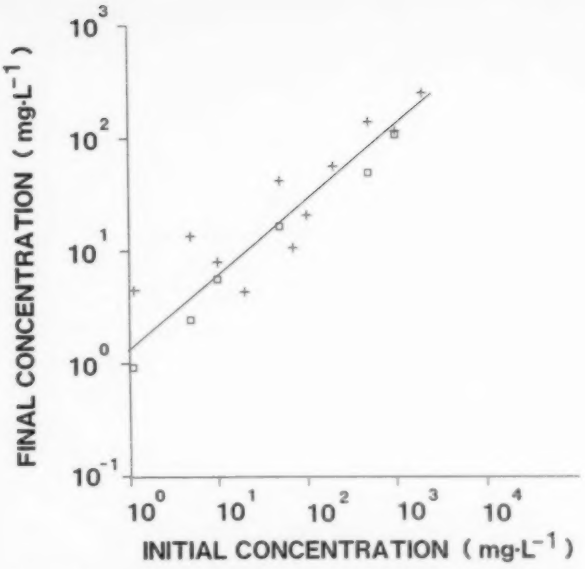

FIG. 5. Changes in suspended sediment concentration after 90-min oxygen consumption measurements in respirometers, either with (+) or without (□) *Mya arenaria*. Initial concentration is based on the weighed sediment added, while final concentration is the amount determined by filtering the suspension after the incubation. The regression is given in the text.

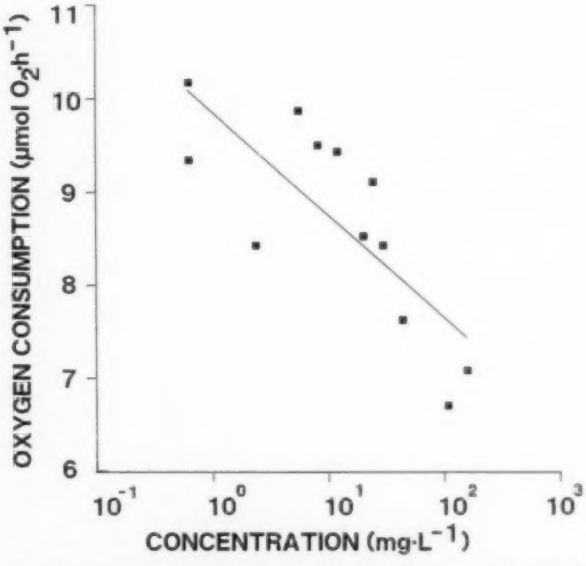

FIG. 6. Oxygen consumption of individual *Mya arenaria* (standardized to a clam 2 g dry weight) measured in the presence of suspended sediment. Concentration is corrected for sedimentation during the incubation according to Eq. 4. The regression is given in the text.

concentration remaining in suspension ($r = 0.899$, $p < 0.001$; Fig. 5):

(4) $\log F = 0.56 \log I + 0.36.$

The presence or absence of clams seemed to make little difference in this relationship (Fig. 5), suggesting that sedimentation rather than clam filtration was controlling particle load in the chambers. This equation was used to correct initial concentrations for settlement loss during the experiment. Oxygen consumption (micromoles per hour) of standard sized clams declined logarithmically with increasing turbidity, despite low initial concentrations of suspended sediments (Fig. 6). This relationship ($r = 0.83$, $p < 0.01$) can be described as

(5) O_2 consumption $= -1.10 \log$ (concentration) $+ 9.86$

where concentration (milligrams per litre) was corrected for sedimentation according to Eq. 4.

In short-term exposures to suspended sediments at initial concentrations up to 852 mg·L^{-1}, pseudofeces production was not observed. At initial sediment concentrations of 1152 mg·L^{-1}, pseudofeces occurred as long strings of mucus-wrapped sediment. In addition to pseudofeces, there were tracks of unconsolidated sediment expelled by the clam. This sediment may have been purged from the mantle cavity or siphon without being handled along the gill or labial palps, as there was no evidence of mucus covering. Initial concentrations of 852–1152 mg·L^{-1} correspond to final concentrations of 100–119 mg·L^{-1} after correcting for sedimentation in the chambers using Eq. 4. These values provide a concentration range for the threshold of pseudofeces production.

Discussion

Mya arenaria responded differently to short- and long-term exposure to suspended sediments. The short-term reaction of clams to increased particle load, even at low concentrations (<20 mg·L^{-1}), was reduced aerobic respiration rate. There are few comparable data for bivalves. Widdows et al. (1979) found no linear relationship between particle concentration and oxygen consumption in *Mytilus edulis*; however, their data indicated reduced respiration in six of nine mussels held in 100–200 mg suspended sediments ·L^{-1} compared with the same animals held at 3–12 mg·L^{-1}. Møhlenberg and Kiørboe (1981) also found no relationship between respiration and concentration in short-term exposures with the surf clam *Spisula truncata*, but rate measurements were made after removing clams from suspended sediments.

Aerobic respiration rates of controls (0 mg·L^{-1}) in our short-term exposures were similar to values determined by Emerson et al. (1988) for *Mya* of similar tissue weight from the Atlantic coast of Nova Scotia. In contrast, oxygen consumption of control clams in our long-term experiment was almost five times higher than determined in the previous study for clams of comparable weight. However, our long-term measurements were made at higher temperatures (8°C) with clams kept in food-rich conditions, in contrast with clams held for several days without food (4.5°C) in Emerson et al. (1988). This difference in respiration most likely resulted from enhanced oxygen consumption which occurs in actively feeding animals (Griffiths and Griffiths 1987). The tissue weight gain of control clams (Fig. 4) was not reflected in an increased respiration rate. However, the allometry of respiration rate (Eq. 3) indicates that tissue growth during the experiment would produce a respiration increase of only ~20%.

Jørgensen et al. (1986) suggested that diffusion of oxygen to suspension feeders should not be limiting except at very low ventilation rates. We did not assess whether the decrease in

aerobic respiration that occurred in the presence of turbidity (Fig. 6) invoked an oxygen debt and anaerobiosis. Whether a decrease in respiration causes or is caused by a lower ventilation rate, the relationship between these variables is well documented for a variety of suspension feeding bivalves (Jørgensen et al. 1986; Griffiths and Griffiths 1987). We thus consider the respiration results of the short-term exposure experiment to indicate that ventilation rate was inhibited by suspended sediments.

Clearance rate (ventilation determined by particle depletion) has been measured in *Mya arenaria* (see Foster-Smith 1975; Møhlenberg and Riisgård 1979), but there are few data on functional changes due to particle concentration. The decrease in respiration with increasing turbidity in our study is analogous to the often observed decline in bivalve clearance at high particle concentrations (Bricelj and Malouf 1984; Griffiths and Griffiths 1987). Although bivalves utilize a variety of means to control ventilation rates, *Mya arenaria* appears to restrict its exhalent siphon intermittently to reduce net ventilation activity at high food concentrations (Foster-Smith 1976). A decrease in time spent pumping rather than a constant lower rate allows reduced water exchange.

Bivalves can also clear high particle concentrations by producing pseudofeces (Foster-Smith 1975). *Mya arenaria* appears to have a high threshold for triggering pseudofeces production compared with other bivalves, but these thresholds vary with the nature of the suspension (Griffiths and Griffiths 1987). In feeding experiments with *Mya arenaria*, Shumway et al. (1985) found no pseudofeces at $3.7 \times 10^5 \cdot mL^{-1}$ cells·mixed phytoplankton culture, a concentration that induced pseudofeces in other infaunal species (*Arctica islandica, Ensis directus*). Kiørboe and Møhlenberg (1981) collected pseudofeces from *Mya arenaria* at suspended sediment concentrations of only 10–20 $mg \cdot L^{-1}$. Our long-term and some short-term exposures were carried out beyond the apparent pseudofeces threshold for these Fundy sediments (100–119 $mg \cdot L^{-1}$), and it is likely that pseudofecal production occurred under such conditions. *Mya* has another mechanism for rejecting material, since tracks of unbound sediment ejecta were found near the shell; in extreme turbidity, clams must expunge material which has sedimented into the mantle cavity or siphon (Stevens 1987).

The reduced clearance rates which apparently occurred in the short-term exposure to turbidity were indicative of the physiological stress which took place in long-term exposures. Consistently high particle loads inhibited food intake, and clams began to starve, catabolizing stored protein as indicated by decreasing O:N ratio. There was no evidence of acclimation to turbidity; the data suggest a greater decrease in respiration during the latter part of the experiment. Unlike the short-term exposures, the reduction in oxygen uptake during the 7-wk experiment was not an instantaneous response to turbidity because the weekly respiration rates were measured in filtered seawater. Instead, declining oxygen uptake indicated the worsening nutritional condition of the exposed animals and a conservative metabolic strategy. We suggest that with continued turbidity stress, aerobic respiration would decline towards a basal metabolic rate (i.e. maintenance level) characteristic of starving animals (Hawkins et al. 1985).

Changes in excretion rate also demonstrated the extent of stress in clams exposed to suspended sediments. Allen and Garrett (1971) found ammonia excretion rates in *Mya arenaria* (starved for >10 d) which were similar to the rates of our exposed treatment, when corrected for the larger size of their specimens. They suggested that excretion of urea was only 10% of rates for ammonia. Although Allen and Garrett (1971) found a leveling of excretion after 10–15 d, their results were based on continuous sampling of holding aquaria where ammonia content was probably influenced by bacterial processes. In our study, absolute excretion rates and a decreasing O:N ratio provided measures of turbidity stress. The range in O:N ratios during our experiment (14–27) was similar to the range determined for sea scallops (*Placopecten magellanicus*) (9–31; Grant and Cranford 1991) but less than that of *Mytilus edulis* (36–355; Bayne 1973). The O:N ratio may have continued to decline due to turbidity, toward a minimum value which reflects pure protein catabolism (Bayne et al. 1985).

In addition to the metabolic measures of stress, growth responses provided evidence of the negative impact of turbidity. Despite an adequate ration of microalgal food in the exposure treatment, there was no mean growth compared with the control. This difference in growth was a result of the quality as well as the quantity of food present. Although organic matter was present in the suspended sediment, including benthic bacteria and microalgae (Cammen 1982), seston concentrations exceeded the level of sediment supplements (~5–8 $mg \cdot L^{-1}$) which may enhance growth in bivalves (Kiørboe et al. 1981; Møhlenberg and Kiørboe 1981). Food quality was essentially diluted by the high inorganic content of the suspension (Widdows et al. 1979).

Soft-shell clams can also select microalgae from a mixed particle assemblage, preferentially rejecting inorganic matter in pseudofeces (Kiørboe and Møhlenberg 1981). Particle size is a less likely basis for this selection, since the dried algae tended to clump and thus had a major overlap with suspended sediments in the 64–128 μm size range. Although *Mya arenaria* shows almost 100% retention efficiency in the 4–7 μm size range (Møhlenberg and Riisgård 1978), the larger paticle size in our experiments was not detrimental because control clams derived nutrition from the microalgae. On an even finer scale, flow cytometry indicated that *Mya* had little ability to select between three microalgal species before ingestion, although some sorting occurred along the digestive tract (Shumway et al. 1985). In our study, despite potential mechanisms to feed in turbid waters, i.e. particle selection coupled with pseudofeces production, *Mya arenaria* was unable to derive adequate nutrition in the 100–200 $mg \cdot L^{-1}$ particle load of the exposure treatment.

Bricelj and Malouf (1984) contrasted the feeding strategies of bivalves in the presence of suspended sediments: species that reduce clearance rate and those that combine pseudofeces and selectivity to optimize ingestion. *Mya arenaria* apparently utilizes both strategies depending on the particle load and duration of exposure. In short-term exposure, *Mya* reduced its respiration rate and presumably its ventilation rate to endure temporarily elevated concentrations of suspended sediment. Such conditions would be frequent on muddy tidal flats, where small waves in shallow water cause resuspension (Anderson 1983). A negative reaction to turbidity is apparent from Fig. 6, and from the retracted behaviour of clams during the first week. In longer term turbidity exposure and/or at higher concentrations, clams pump intermittently (Foster-Smith 1976) and produce pseudofeces to decrease their burden of inorganic material. This strategy is only successful when the ingestion rate (clearance × concentration) and the organic quality are both high enough to offset reduced pumping in a concentrated inorganic suspension. The extended siphon apparent in the latter part of our

experiments may have been an attempt to filter water beyond a perceived near-bottom turbidity maximum.

The amount of material in suspension over mudflats is influenced by a variety of factors including resuspension, ice, precipitation, bioturbation, input from channels and rivers, and even clam digging (Anderson 1983; Anderson and Mayer 1984). Suspended sediment concentration over several mudflats in the Bay of Fundy averages 200–500 $mg \cdot L^{-1}$ (Hargrave et al. 1983) but may reach 3000 $mg \cdot L^{-1}$, depending on wind conditions (P. Cranford, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.). Alteration of flow due to a tidal barrage may enhance intertidal deposition leading to a near-bottom fluid mud or nepheloid layer (Amos 1984). Rather than being permanently deposited, this material will be resuspended during various stages of the tide. As a result of causeway construction in the Bay of Fundy, huge tidal flats of low-density mud have been deposited at rapid rates (metres per year; Turk et al. 1980; Daborn and Dadswell 1988). The Annapolis Basin has relatively low turbidity (Daborn and Dadswell 1988), but attempts to reduce the permeability of the causeway with clay may have introduced a new source of fine sediments to the adjacent tidal flats. Recently deposited fine sediments with high water content and limited consolidation will have a low shear strength (Mehta 1989), resulting in permanent near-bottom turbidity.

Mya arenaria is not sensitive to burial under sand or consolidated mud (Emerson et al. 1990), but our results and previous anecdotal information (Newcombe 1935; see also references in Appledoorn, 1983) indicate that turbidity resulting from a mobile mud layer would be detrimental to growth. At prolonged concentrations >100 $mg \cdot L^{-1}$ (>2 wk), *Mya* displayed depleted nutritional state and reduced growth rate. In turbidity experiments, rough appearance of the siphon suggested greater susceptibility to microbial infection (Kraeuter and Castagna 1989). The associated lethargic behaviour may have consequences for clam burrowing and/or predator avoidance. These negative effects will probably result in increased mortality, decreased reproductive output, and a decline in the fishery. Additional studies of intertidal sediment transport and suspended particle loads in relation to the settlement and growth of *Mya arenaria* will allow further application of our results to natural populations.

Acknowledgements

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Phosphorus Pool Sizes and Fluxes in the Epilimnion of a Mesotrophic Lake

William D. Taylor and David R. S. Lean

Department of Biology, University of Waterloo, Waterloo, Ont. N2L 3G1, Canada
and
National Water Research Institute, Canada Centre for Inland Waters, P.O. Box 5050, Burlington, Ont. L7R 4A6, Canada

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A plausible budget for phosphorus fluxes between different size/functional groups was constructed for the early summer epilimnetic plankton of Jacks Lake, Ontario. Consideration of the complex interactions at the base of the food web, particularly the ability of nanoplankton to graze on picoplankton, helped to resolve the question of phosphate uptake by bacteria versus algae. While the concentration of phosphate we estimated with column chromatography was lower than chemical detection limits, it was still too high to reconcile with our other measurements and we conclude that actual phosphate concentrations may be less than 1 nM. Some microplankton were shown to liberate dissolved phosphate and organic phosphorus when lake water is filtered. The phosphorus content and turnover of major ciliate, rotifer, and crustacean zooplankton were determined and the importance of these compartments as phosphorus sources and sinks was included in the epilimnetic model.

Nous avons élaboré un bilan plausible des flux de phosphore entre différents groupes de taille et de fonction pour le plancton épilimnique du début de l'été dans le lac Jacks, en Ontario. La prise en compte des interactions complexes qui se situent à la base du réseau trophique, particulièrement l'aptitude du nanoplancton à brouter le picoplancton, a aidé à résoudre la question de l'absorption du phosphate par les bactéries par comparaison aux algues. Si la concentration de phosphate estimée par chromatographie sur colonne était inférieure aux limites de la détection chimique, elle restait trop élevée pour concorder avec nos autres mesures, et nous en concluons que les concentrations réelles de phosphate peuvent être inférieures à 1 nM. Nous avons observé que certains microplanctontes libèrent du phosphate et du phosphore organique dissous lorsque l'eau du lac est filtrée. La teneur en phosphore et le taux de renouvellement des principales espèces de ciliés, de rotifères et de crustacés zooplanctoniques ont été mesurés, et nous avons inscrit dans le modèle de l'épilimnion l'importance de ces éléments comme sources et drains de phosphore.

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Research on the phosphorus (P) cycle of lakes has been a major focus for limnologists, largely because of its importance to the problem of eutrophication. However, the topic has gained broader relevance because the partitioning and flux of P in lakes can serve as a template for our understanding of other limnological processes. That is, P is a useful "currency." This is especially true as studies of the P cycle expand from a focus on fluxes within the epilimnion to whole lakes (e.g. Levine et al. 1986) and to interlake comparisons (e.g. Mazumder et al. 1990b).

Despite the wealth of existing information on the P cycle in the epilimnion of temperate lakes, there are still gaps in our understanding which are revealed when models of components of the system are assembled. Some of these problems areas were set out clearly by Tarapchak and Nalewajko (1986) in their introduction to the Phosphorus Dynamics Symposium held at Brock University in St. Catharines, Ontario. Perhaps the most basic problem is that the concentration of phosphate (and of other components of dissolved P) cannot be quantified at the low levels typical of P-limited lakes. Soluble reactive phosphorus (SRP) greatly overestimates phosphate at low concentrations.

A second problem is that bacteria, usually considered to be important mineralizers, dominate the uptake of phosphate in most lakes with low concentrations of SRP (Rigler 1973; Currie and Kalff 1984; Berman 1985; Lean et al. 1987a). This paradox begs the question of how algae obtain P and why bacteria take up more inorganic nutrient than the primary producers (Currie and Kalff 1984). Dissolved organic phosphorus (DOP), if it is used largely by algae rather than by bacteria, might be the answer (Currie et al. 1986), but the release of phosphate from phosphomonoesters, which is the mechanism of DOP use that algae might dominate, may be relatively small (Heath 1986).

The role of zooplankton in the P cycle is also problematical. Although zooplankton do certainly regenerate P in quantitatively important amounts, removing or concentrating them does not seem to have the expected impact on phosphate dynamics (Hamilton and Taylor 1987; McCauley and Kalff 1987). The suggestion that phagotrophic protozoa may be the important mineralizers is appealing, but culture studies have found them to be rather parsimonious users of resources, as likely to behave as nutrient sinks when those nutrients are rare (Gude 1985; Taylor 1986; Caron et al. 1988). Similar results have been obtained for crustacean zooplankton (Olsen et al. 1986).

Among the underlying causes of these controversies has been our lack of knowledge concerning the trophic relationships among many of the important organisms involved, i.e. a lack of "natural history" for plankton (Smetacek and Pollehne

1986). This ignorance of natural history may also underlie our inability to balance carbon budgets for the Laurentian Great Lakes (Scavia et al. 1986; Lean et al. 1987b; Scavia 1988).

New information on the interactions of algae, zooplankton, and bacteria is shedding important light on these contradictions. Examples include the consumption of bacteria by algae (Bird and Kalff 1986; Porter 1988), the excretion of carbon (C) by algae (Bjornsen 1988) and its uptake by bacteria (Coveney 1982; Søndergaard et al. 1985), and the importance of autotrophic picoplankton (Kimmel 1983). Other suggestions, such as the importance of DOP as a P source for algae (Currie and Kalff 1984; Chrost and Overbeck 1987), the production of nutrient patches by zooplankton (Lehman and Scavia 1982), opportunistic uptake of phosphate (Lean et al. 1987a), and the temporal disequilibrium between dissolved organic C production and utilization (Hecky and Fee 1981; Scavia and Laird 1987), are difficult to quantify and less widely accepted.

The studies cited above have largely concentrated on parts of the P cycle. Seldom have data on dissolved P fractions and their dynamics been collected simultaneously with data on the biomass and dynamics of particles. Therefore it is uncertain whether we are now in a position to create a feasible model of P cycling within the epilimnion. Further, such a model might shed some light on which of these suggestions are necessary or sufficient to accommodate the observed pool sizes and fluxes.

The purpose of this paper is to describe the P cycle in the epilimnion of Jacks Lake, Ontario. It contains a variety of data on the P fluxes and pools in the epilimnion, determined over several years (1984–87), but always at the same sampling station and at approximately the same time of year (24 June to 3 July). Some of these data are novel, for example those on the breakage of delicate microzooplankton and their contribution to error in estimating dissolved P, and the actual measurements of P in individual zooplankton and their contribution to particulate P. Other measurements, such as ingestion and excretion rates of zooplankton, have been made before but are uniquely complete here in their coverage of the zooplankton community. Experimental studies of the P cycle also have been carried out at this site (Hamilton and Taylor 1987; Bentzen 1986), and there are relevant earlier studies from which we can draw (Taylor and Lean 1981; Lean and White 1983; Pick et al. 1984; Bentzen 1990; Bentzen and Taylor 1991a; T. R. Fisher and D. R. S. Lean, unpubl. data). We weld these data into a budget model for the epilimnion, to address whether inconsistencies actually exist among the measured pool sizes and fluxes, and, if so, where the error in measurement or concept lies. We show that by considering new information on relationships at the base of the plankton food web, a plausible synthesis of the data can be achieved.

Materials and Methods

Filter-fractionation (0.2, 0.8, 3.0, and 12 μm) of total phosphorus (TP) and ^{32}P used 47- and 25-mm Nuclepore polycarbonate filters, respectively. Larger size-classes (41 and 200 μm) were collected on Nitex nylon screens. P was measured as phosphate using the ascorbic acid method after hydrolysis of organic P with persulfate in an autoclave (Parons et al. 1984). Phosphate was measured using column chromatography of ^{32}P-labelled lake water filtrate (0.2 μm) with Sephadex G25 resin (Taylor and Lean 1981). The column was calibrated with $^{32}PO_4$ and blue dextran.

Abundance and biomass of crustaceans were estimated from Schindler–Patalas samples. Abundance was converted to biomass using published length–weight regression equations. Abundance of ciliates and rotifers was estimated from samples collected with a Van Dorn bottle, fixed in 1% Lugol's iodine, and settled for examination with an inverted microscope.

The P content of individual zooplankton species was estimated in one of three ways. For crustaceans, individuals were picked live from net hauls with forceps and placed in vials at 5–50 per vial depending on size. For rotifers, individuals were collected with micropipets, washed in deionized and distilled water, and placed in vials. The volume in the vials was made up to 35 mL with deionized and distilled water, and P was estimated as above. For ciliates, individuals that had been in ^{32}P-labelled lake water for at least 48 h were washed and their ^{32}P content assayed (see below). Their P content was estimated assuming isotopic equilibrium with the lake water. That is, P content of a cell was estimated as the ^{32}P per cell divided by the ^{32}P concentration in the water multiplied by the TP concentration in the water. Previous work with assimilation of ^{32}P by ciliates (Taylor and Lean 1981; Taylor 1984) indicates that most have stopped increasing in ^{32}P content by 48 h, which is about two doubling times. Similar estimates were obtained by applying a value of P content per cell volume from a laboratory study of P metabolism of ciliates (Taylor 1986).

Phosphate uptake by plankton was measured by placing 50 mL of lake water in a polyethylene beaker and adding about 10 MBq of carrier-free [^{32}P]phosphate. Filtration of several 1-mL aliquots on 0.2-, 0.8-, 3.0-, and 12-μm filters was used to assess translocation of ^{32}P to various particulate fractions. Uptake constants for phosphate were calculated as the slope of a plot of the logarithm of the fraction of ^{32}P remaining in solution (passing a 0.2-μm filter) versus time.

The rate of hydrolysis of phosphomonoesters was estimated by adding methyl fluorescein phosphate (MFP) to lake water and observing the rate of increase in fluorescence with time. As the substrate is not added at tracer amounts, the rate of hydrolysis of MFP was expressed as percent per hour at several concentrations of added MFP and extrapolated to zero to estimate the rate of hydrolysis of MFP at the ambient concentration of phosphomonoesters.

Ingestion (assimilation plus release) of P by zooplankton was measured as in Taylor (1984). Briefly, assimilation of P was assessed by adding carrier-free [^{32}P]phosphate to lake water in 1- to 2-L polyethylene bottles. Zooplankton were removed singly, washed, and assayed for ^{32}P over about 48 h. For some crustaceans, assimilation rates were calculated for two size-classes or as a function of size (*Daphnia*). Release of P was estimated by washing animals that had been in labelled lake water for at least 48 h and placing them into 1–2 mL of unlabelled lake water in embryological watchglasses. The release of ^{32}P into the water during 20 min of incubation was used as an estimate of P release rate. Ingestion rates were expressed as the sum of the rates at which ^{32}P was accumulated and released, assuming that both were in isotopic equilibrium such that ^{32}P radioactivity can be converted to weight of P by multiplying it by the TP concentration in the water and dividing it by the concentration of ^{32}P radioactivity in the water. To convert rates of P ingestion into grazing rates for comparison with grazing chamber estimates (below), ingestion rates were divided by the concentration of particulate (0.2–41 μm) P.

Grazing rates for zooplankton were obtained using a 1-L grazing chamber. Food labelled with ^{32}P was prepared by add-

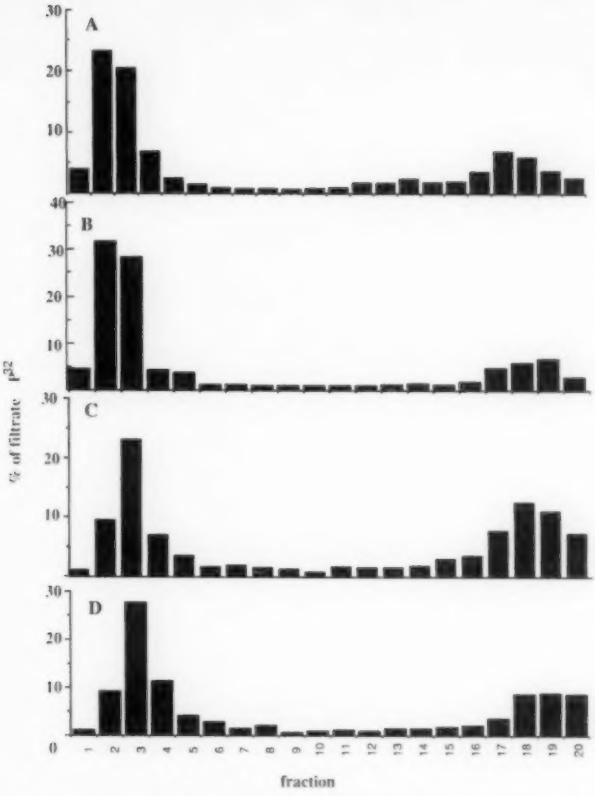

FIG. 1. Column chromatography of lake water filtrates (0.2 μm) from Jacks Lake. The horizontal axis indicates the fractions in the order in which they were eluted from the column, while the vertical axis indicates the fraction of the total radioactivity eluted in that fraction. (A and B) Lake water labelled for 48 h with carrier-free [^{32}P]phosphate in 1984 and 1986. (C) Unlabelled lake water into which labelled *Strombidium viride* were added immediately prior to filtration. (D) same, except that *Strobilidium velox* were used. Peaks at fractions 1–5 and fractions 17–20 correspond to elution of compounds >5000 molecular weight (void volume) and [^{32}P]phosphate.

ing about 30 MBq [^{32}P]phosphate·mL prefiltered (20 μm) lake water^{-1} along with about 0.15 μM phosphate and incubating for at least 1 h. The added phosphate causes the label to become distributed among size-classes in a manner similar to TP (Mazumder et al. 1990a). The grazing determinations were done with 10-min exposures to the labelled food. Grazers were collected on 41-μm screens.

Results

Size and Dynamics of Dissolved P Fractions

The concentration of phosphate in lake water, as determined by column chromatography of ^{32}P-labelled lake water filtrate, was about 19 nM or about 27% of the soluble P in 1984 (Fig. 1A). In 1986, a similar 20% of soluble P eluted from the column as phosphate (Fig. 1B), but soluble P was a smaller fraction of TP, and therefore, phosphate was estimated as only about 6 nM. (Detection limits for phosphate by chemical means are about 10 nM or more.)

TABLE 1. Release of ^{32}P to the filtrate (percent) on filtering ^{32}P-labelled zooplankton in lake water, and the P content (pM) of the protozoa estimated from their ^{32}P content assuming isotopic equilibrium with the lake water. Filters were 0.2-μm polycarbonate (PC) or 0.45-μm cellulose (CE).

	% loss		P content			
Taxon	Range	Mean	Range	Mean	*n*	Filter
Strombidium	52–83	69	1.4–2.7	2.2	3	PC
Strombidium	41–56	49	1.9–3.0	6.1	3	CE
Strobilidium	32–56	48	3.6–7.3	5.1	3	PC
Epistylis (colonies)	3.6–5.7	4.7	30–45	37	2	PC
Asplanchna	6.4–3.6	5.0	18.5–20	19.3	2	PC
Polyarthra	45–76	70	1.1–8.2	3.6	3	PC
Daphnia	0.2–7.4	25	7.6–4369	2461	3	PC

TABLE 2. Size distribution of TP and ^{32}P(0.25 and 48 h after addition of ^{32}P-labelled phosphate) by filter-fractionation. Each data point is the mean of duplicates.

	1986		1987		1986 (^{32}P)	
Size fraction (μm)	nM	%	nM	%	0.25 h	48 h
<0.2	117	30.4	94	28.3	1.3	23.7
0.2–0.8	114	29.6	42	12.7	63.2	42.1
0.8–3.0	0.0	0.0	18	5.4	27.3	16.4
3.0–12	38	10.0	19	5.6	5.3	5.0
12–41	16	4.2	82	24.8	2.9	12.8
>41	99	25.8	77	23.2		
Total	384		332			

To investigate whether some of this dissolved material might be generated by the breakage of delicate zooplankton, we placed zooplankton that had been in ^{32}P-labelled lake water for 48 h into unlabelled lake water and filtered them. The amount of label appearing in the filtrate varied among the organisms we tried; some, like *Daphnia*, retained their label, while others, especially choreotrich ciliates, released most of their radiolabel to the filtrate (Table 1). The material released to the filtrate was also subjected to column chromatography and appeared to consist of both phosphate and high molecular weight P compounds (Fig. 1C). Note that this effect of filtration on release of ^{32}P-labelled material uses zooplankton that have been incubated with label for a relatively long period. In a 15-min radiobioassay of phosphate uptake, the zooplankton would have assimilated an insignificant amount of label and their release of that label would not be detectable.

The uptake rate constant for phosphate was variable: 4.6·h^{-1} in 1984, 18.0·h^{-1} in 1986, and 12.8·h^{-1} in 1987. These values provide estimates for phosphate turnover time of 13.0, 3.3, and 4.7 min. Because the apparent size of the phosphate pool was larger in 1984 than 1986, the estimates of phosphate flux obtained by combining these rates with the phosphate estimates from the chromatograms above are similar, 0.09 and 0.12 μM·h^{-1}. These ignore the contribution of broken organisms to the apparent size of the phosphate pool, so they are probably overestimates. Uptake was dominated by bacteria-size particles; most of the label retained by a 0.2-μm filter could pass a 0.8-μm filter, and little was retained by a 12-μm filter (Table 2).

MFP was quickly hydrolyzed in Jacks Lake water, indicating the presence of alkaline phosphatase. Because this substrate is

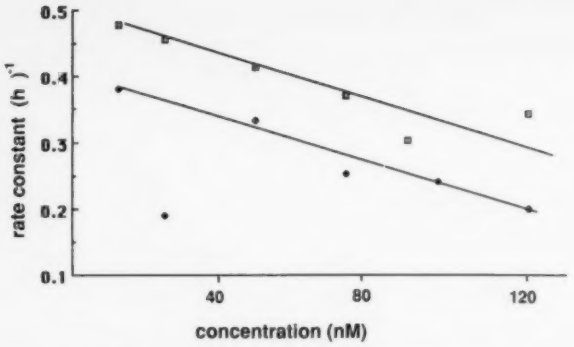

FIG. 2. Rate of hydrolysis of MFP as a function of concentration. The *y*-intercepts estimate the rate of hydrolysis of phosphomonoesters under ambient conditions.

TABLE 3. Size distribution of alkaline phosphatase activity (the rate formation of methyl fluorescein from 130 nM MFP) in prefiltered lake water. Units are $nM \cdot h^{-1}$. Ranges given are for three determinations.

Size fraction (μm)	Activity	% of total
<0.2	6.9–9.8	21–25
0.2–0.8	2.2–9.0	7.6–23
0.8–3.0	6–11	21–28
3–12	1–9	3–9
>12	5–10	17–26
Total	20–40	

not added in tracer quantities, the relationship between substrate added and apparent hydrolysis rate was plotted (Fig. 2) and extrapolated back to zero addition. Resulting estimates of hydrolysis rates for equivalent phosphomonoesters in these two experiments were 0.40 and $0.49 \cdot h^{-1}$. If all DOP (approximately 0.1 μM, Table 2) was substrate for alkaline phosphatase, the amount of P supplied could be 0.4–0.5 $\mu M \cdot h^{-1}$.

Filter-fractionation studies indicated that much of the phosphatase activity is by particles (Table 3) and therefore could be tightly coupled to uptake of the hydrolyzed phosphate. Over three experiments, 17–26% of the activity was due to particles retained by a 12-μm filter, and 31–50% was due to particles passing a 12-μm filter but retained by a 0.8-μm filter. However, 21–25% of the activity passed a 0.2-μm filter, and if this component is truly dissolved, it is probably creating dissolved phosphate.

Particulate P

The distribution of P among size-classes (Table 2) differs from the size distribution of assimilated ^{32}P. The dissolved fraction is a similar fraction of the total, but the particulate fraction is less concentrated in the smaller size-classes. In 1987, there was about twice as much in algal-sized (3- to 12-μm) particles and half as much in bacteria-sized (0.2- to 3.0-μm) particles. In both years, about 25% of the P, or one third of the particulate P, was in particles retained by a 41-μm screen.

Zooplankton in the epilimnion of Jacks Lake are relatively small; mean length of crustaceans was 0.57–0.69 mm on five different occasions on which they were quantified, even though *Daphnia* were abundant. Biomass of crustaceans ranged from 37 to 213 $\mu g \cdot L^{-1}$ and averaged 99 $\mu g \cdot L^{-1}$. If P is about 1.2% of dry weight (assuming 41:1 P:C by weight and that C is one half of wet weight), then crustacean P would range from 0.014 to 0.082 μM and average 0.038 μM. The P contents of crustaceans and rotifers estimated directly, when combined with the abundance estimates from 1986, produce an estimate of crustacean P of 0.06 μM (Table 4). Including ciliates increases the estimated zooplankton component of TP to 0.063 μM. Most of the zooplankton P is in the larger herbivores (calanoids and *Daphnia*).

By comparing Tables 2 and 4, one would infer that much of the >41-μm fraction of TP is zooplankton, but coarse algae are also represented. Large colonial algae, especially *Gloetrichia*, are abundant in Jacks Lake. Individual colonies were assayed for P as for zooplankton and found to contain 2.2 $pM \cdot colony^{-1}$.

Movement of P through Zooplankton

Zooplankton had uptake rates for P from 0.14 to almost 190 $\mu M \cdot h^{-1}$, with rates increasing with body size both across species and within *Daphnia* (Table 5). These can generate estimates of individual filtering rate by dividing them by the amount of particulate P in particles which can be used by grazers (0.2–41 μm, about 0.164 μM, Table 2). Assimilation rates were not provided for predators because they would be underestimated for species whose prey is not labelled quickly on addition of ^{32}P (Taylor 1984). The fraction of that P which was released varied around 50%, with small *Daphnia* apparently having the lowest assimilation efficiencies.

When these rates are multiplied by abundances and summed across species, they predict community grazing rates of 1570 $pM \cdot h^{-1}$ (Table 5). Multiplying this rate by 24 h to create a daily rate, and dividing this by the amount of P in the 0.2- to 41-μm fraction (0.164 μM), predicts a community filtering rate of $23\% \cdot d^{-1}$ on that fraction. Community grazing rates measured with the grazing chamber for particles less than 20 μm were similar, 27.1 and $24.5\% \cdot d^{-1}$ in 1986 and 1987.

P Budget for Jacks Lake Plankton

With the P quantities and rate measurements described above, we attempted to complete a balanced budget for P movement between compartments in the epilimnion of Jacks Lake (Fig. 3A). Compartments were assigned P contents according to Tables 2 and 4. We assigned an uptake rate constant of $6 \cdot h^{-1}$ to the dissolved in organic phosphorus (DIP) pool, which is intermediate relative to the values we observed, with most of that flux ($5 \cdot h^{-1}$, Fig. 3B) going initially to picoplankton (0.2–3 μm), as indicated by the distribution of ^{32}P at 0.25 h (Table 2).

P flux to microplankton was estimated as the sum of the uptakes rates for ciliates, rotifers, nauplii, and *Bosmina* (Table 5). P flux to herbivorous mesoplankton was estimated as the sum of the uptake rates for calanoids and *Daphnia*. Excretion by zooplankton and fish was assumed to be 50% of ingestion, which is an approximation based on Table 5. All P assimilated by microplankton was transferred to invertebrate carnivores as predation. All P assimilated by mesoplankton herbivores and invertebrate carnivores was transferred to fish. These quantities, directly or indirectly supported by measurements, are illustrated in Fig. 3B. Given the natural variability in the parameters, the different times at which they were meas-

TABLE 4. Abundance of ciliates, rotifers, and crustaceans in Jacks Lake plankton collected at 5 m in 1986 (individuals per litre), their P content (pM), and the product of these (nM).

Taxon	Length class (μm)	Abundance	P content	Product	Group totals
Ciliates	0–20	7000	0.38	2.71	
	20–30	2420	0.65	1.55	
	>30	1710	1.68	2.87	7.13 (11%)
Rotifers	Polyarthra	200	23.6	4.71	
	Keratella	210	29.5	6.19	
	Other	100	25.8	2.58	13.48 (21%)
Cyclopoids	<700	3.66	1219	4.35	
	>700	1.50	3064	8.97	8.97 (14%)
Calanoids	<700	2.53	1090	2.77	
	>700	5.74	3039	17.45	20.23 (31%)
Daphnia	500–600	0.73	368	0.27	
	600–700	0.80	558	0.45	
	700–800	1.00	787	0.79	
	800–900	1.00	1064	1.06	
	900–1000	0.33	1390	1.85	
	1000–1100	0.87	1761	1.53	
	1100–1200	0.87	2184	1.90	
	1200–1300	0.67	2658	1.78	
	1300–1400	0	3138	0	
	1400–1500	0.43	3761	1.62	
	1500–1600	0.53	4393	2.33	13.58 (21%)
Bosmina		1.00	161	0.16	
Chydorus		3.27	161	0.53	
Diaphanosoma		1.7	123	0.21	0.90 (0%)
Total				64.23	

TABLE 5. P assimilation (A), release, and uptake (U) rates and the ratio between assimilation and uptake for Jacks Lake zooplankton. All are expressed as $pM \cdot h^{-1}$. Numbers in parentheses are standard errors and sample size, except for *Daphnia* for which the coefficient of determination for the relationship between length and rate and the sample size are provided.

	Individual				Population		
Taxon	Assimilation	Release	Uptake	A/U	Abundance	Uptake	Release
Strombidium	0.14 (0.01, 8)	0.10 (0.02, 11)	0.25	0.58	1710	428	171
Strobilidium velox	0.24 (0.02, 4)	0.17 (0.01, 3)	0.40	0.57	90	36	15
Keratella	0.26 (0.03, 10)	0.20 (0.02, 10)	0.46	0.57	210	97	42
Polyarthra (sp. 1)	0.06 (0.01, 3)	0.13 (0.03, 3)	0.19	0.31	150	28	20
Polyarthra (sp. 2)	0.20 (0.01, 17)	0.23 (0.05, 6)	0.43	0.47	50	21	11
Gastropus	0.07 (0.01, 14)	0.07 (0.01, 10)	0.14	0.49	50	7	4
Asplanchna		1.3 (0.67, 3)			10		13
Daphnia							
462 μm	8.2	3.1	38.3	0.21			
800 μm	42.2	42.0	84.2	0.50			
1200 μm	82.4	60.0	142.4	0.60			
1572 μm	120.0	69.1	189.1	0.63			
Total	(0.96, 6)	(0.81, 11)			8.2	875	391
Bosmina	3.15 (0.44, 7)	3.60 (0.72, 7)	6.7	0.47	1.0	7	4
Ceriodaphnia		8.01 (4.6, 3)			3.0		24
Chydorus		2.01 (1.1, 3)			3.30		7
Cyclopoids		1.16 (0.54, 5)			5.16		6
Calanoids	2.79 (0.18, 18)	4.10 (0.68, 11)	6.9	0.40	8.27	57	34
Nauplii	0.77 (0.08, 21)	0.62 (0.12, 12)	1.4	0.55	10.0	14	6
Total						1570	748

ured, and the error in the measurements, the values assigned are round numbers within the observed ranges and are meant only to illustrate whether major inconsistencies exist.

Working from Fig. 3B, and attempting to balance the budget, a problem arises. The flow of phosphate into picoplankton, given our measured uptake constant and the lowest estimates for DIP concentration, is much too high. If phosphate was 6–19 nM, as we measured by column chromatography (Fig. 1), the estimated flux would replace the picoplankton pool approximately once per hour, which is too fast in light of grazing

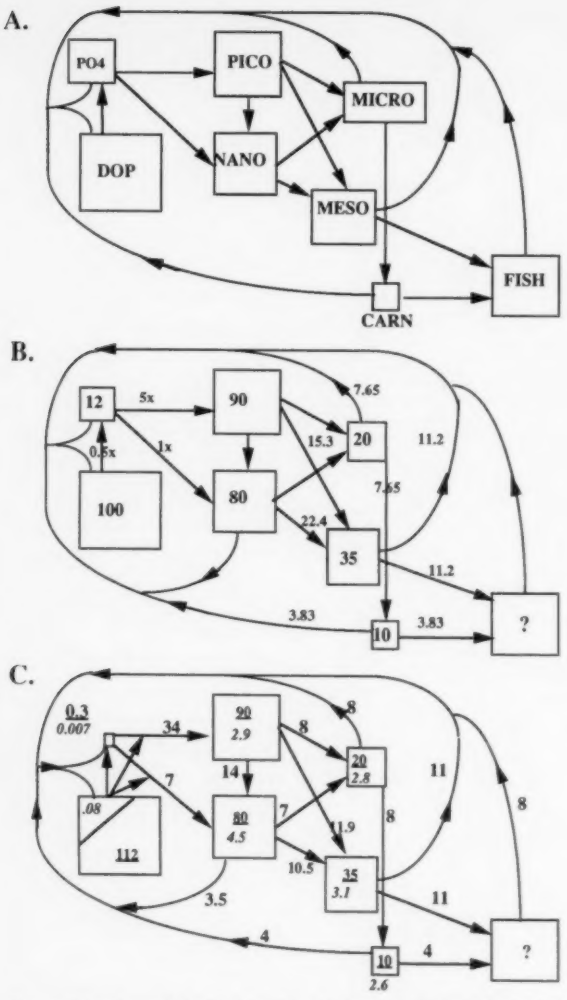

FIG. 3. Simple model of P dynamics in the epilimnion of Jacks Lake. (A) Conceptual framework (size/functional compartments) considered in this study. (B) Pool sizes (nM) and flux rates (nM·d^{-1}, or, for phosphate, uptake constants, h^{-1}) approximately as measured in this study. (C) Plausible budget model derived from the data in Fig. 3b by fitting the phosphate pool size to balance grazing fluxes given the uptake constant for phosphate. The underlined numbers associated with the boxes are the pool sizes, while the italicized numbers are the turnover times for those pools in days. The remaining numbers are daily fluxes.

estimates. Nor could we account for the origin of that P from zooplankton regeneration. Knowing that cell breakage contributes to an overestimation of the DIP pool, we back-calculated what the phosphate pool should be to satisfy grazing losses by pico- and nanoplankton, assuming that there is no other source of P for these groups. This calculation indicates that a phosphate concentration of only about 0.3 nM, well below what we measured, or could be measured by our techniques, would be adequate to supply enough P to balance grazing given an uptake constant of 6·h^{-1}. Of course, if exchange of P occurs between nano- and picoplankton and the dissolved P pool, then our uptake constant is a measure of gross rather than net uptake, and the pool size for phosphate could be larger.

To complete the flow diagram, we partitioned the grazing by micro- and mesozooplankton between picoplankton and nanoplankton. Given that most phosphate uptake is by picoplankton, grazing rates must also be directed primarily at picoplankton. However, the available data suggest that, if anything, grazing should be heavier on nanoplankton. To avoid having to allow zooplankton grazing to be directed primarily at picoplankton, we allowed nanoplankton to graze on picoplankton. We allowed nanoplankton to release only 25% of the P they obtained by phagotrophy in recognition of the fact that some of the organisms responsible are mixotrophs, and so might retain P rather than regenerating it. In the absence of data on the selectivity of grazing by micro- or mesoplankton, or on grazing rates of nanoplankton on picoplankton, we assumed that micro- and mesoplankton herbivores cleared both particle size-classes at equal rates and were then able to solve for the unknown flux from pico- to nanoplankton and complete a budget (Fig. 3C). Turnover times for all of the pools were obtained by dividing pool sizes by inputs. For zooplankton, we included only P that was assimilated in estimating turnover times. Except for phosphate, whose turnover time was measured directly, the turnover times for the pools were similar, from 2.6 to 4.5 d.

To complete a balanced P cycle, we allowed fish to accumulate P at 7.5 nM (about 2% of dissolved and seston P)·d^{-1}, so an equivalent input to the system would be required to balance the budget. If sedimentation was to be considered, again, an additional input would be required or the biomass of the system could be allowed to decrease. Sedimentation from the epilimnion of lakes is also about 1–2%·d^{-1} and is derived primarily from the larger particle classes (e.g. Mazumder et al. 1989). Including sedimentation in our budget would best be accomplished by (very slightly) increasing the turnover rate of the nanoplankton pool to allow it to contribute to sedimentation and by reducing the amount of predation on zooplankton and putting that difference to sedimentation.

The model considers the phosphate and phosphomonoester pools together as the source of P assimilated by pico- and nanoplankton and released by zooplankton. A detailed treatment of the relative magnitudes and dynamics of these sources has recently been produced (Bentzen 1990); both pools are small, turn over rapidly, and are quantitatively important.

Discussion

The description of P distribution among size classes provided above, and the measurements of its movement between those size-classes, can be reconciled into a plausible budget if phosphate concentration is lower than was measured by column chromatography of lake water filtrate, and as long as nanoplankton are allowed another P source, possibly picoplankton. That phosphate is overestimated is supported by the measurements of P released by microzooplankton on filtration. We concur with the suggestion that actual concentrations of phosphate in lake water with rapid phosphate turnover are much lower than can be measured directly by current methods and that some of the phosphate detected by chemical methods or column chromatography is generated in the filtration step of sample preparation.

The Rigler bioassay (Rigler 1966; Tarapchak and Herche 1988) is a means of providing upper limits on phosphate concentration in lake water, actually the sum of the phosphate concentration and the half-saturation constant for its uptake

(Tarapchak and Herche 1988; Bentzen and Taylor 1991a) using the kinetics of [^{32}P]phosphate uptake. Estimates of phosphate concentration of 1–10 nM or less are obtained with this method (e.g. Tarapchak and Rubitschun 1981; Currie 1986; Levine et al. 1986), and similar results have been obtained with this method for Jacks Lake (Pick et al. 1984; Bentzen and Taylor 1991a). The latter also provide evidence for phosphate release on filtration from data on P kinetics in prefiltered samples. Using dialysis rather than filtration to separate particulate and dissolved phases, still lower estimates of phosphate in Jacks Lake have also been obtained with column chromatography (0.3–1.9, mean 1.1 nM; T. R. Fisher and D. R. S. Lean, unpubl. data).

An alternative view from the one we have used to create our budget might be that the phosphate uptake constants measured are estimates of gross uptake and that net uptake is lower and less dominated by picoplankton. This would allow a larger estimate of the phosphate pool. Evidence against a major difference between net and gross uptake includes the observation that ^{32}P cannot be easily displaced from plankton organisms by phosphate (Brown and Button 1979; Grillo and Gibson 1979; Lean and Cuhel 1987), suggesting that assimilated P is not exchanged with extracellular pools. Also, the size distribution of ^{32}P assimilated as [^{32}P]phosphate remains approximately stable for hours to days in Jacks Lake (unpubl. data; Bentzen 1990) as it does in other lakes (e.g. Taylor 1984; Levine et al. 1986). The rapid labelling of DOP appearing in lake water filtrates after addition of [^{32}P]phosphate to lake water (Lean 1973) could reflect rapid exchange, but may also reflect loss of P from cells on filtration or phosphate complexation with organic compounds external to, but associated with, cells. Clearly, the problems of whether net is much less than gross uptake or whether phosphate is much less than SRP are complimentary explanations of the observations, and both are likely to be true to a degree. However, we believe the available evidence supports the contention of our model, that net is close to gross uptake and that phosphate is much less than SRP.

Direct release of P from cells was postulated originally to explain observed radiotracer kinetics, but with each technological advance in measuring phosphate, the concentration became less, so the exchange that was required to produce a reasonable estimate of particulate P turnover diminished as well (T. R. Fisher and D. R. S. Lean, unpubl. data). However, studies on P kinetics of algal cultures provide strong evidence that exchange does occur (Lean and Nalewajko 1976). Therefore, that our estimate of phosphate concentration in Jacks Lake from the budget exercise (0.3 nM) is lower than the newest estimates from bioassays and chromatography following dialysis is to be expected. Of course, the concentration of phosphate in Jacks Lake is almost certainly variable, even in the summer epilimnion, and the values discussed here (<1 to a few nM) may all be encompassed by this variation. We discuss them not as alternatives but as independent arguments that phosphate concentration is of this order, rather than the 10 nM or more that can often be detected in filtered lake water.

We have supplemented the P uptake by nanoplankton by allowing them to graze on picoplankton. The budget could also be plausibly satisfied by invoking direct uptake of DOP by nanoplankton. Given that alkaline phosphatase activity results in hydrolysis of 45% of the available substrate per hour, that about 35% of that activity is due to nanoplankton-sized particles, then we need postulate only a very small pool of phosphomonoesters to offset grazing on nanoplankton. These are both acceptable hypotheses, in that they can balance our P budget. We have emphasized the phagotrophic pathway for two reasons. First, although either mechanism can balance the P budget, it is difficult to reconcile the dominance of bacteria in P flow if they are not growing quickly. Given that metazoan zooplankton do not have higher grazing rates on picoplankton than nanoplankton, it is consistent that the extra grazing on picoplankton comes from nanoplankton. Also, in order to explain the results of earlier experiments on zooplankton manipulations of P kinetics, that removal of zooplankton does not decrease turnover time for phosphate and increase the dominance of bacteria, it is useful to assume that bacteria sustain higher grazing than nanoplankton because of grazers within the nanoplankton and microplankton size-classes (Hamilton and Taylor 1987). Second, recent work using tracer additions of phosphomonoesters suggests that their uptake in Jacks Lakes is more rapid than MFP experiments suggest, but not strongly dominated by nanoplankton at ambient concentrations (Bentzen 1990; Bentzen and Taylor 1991b).

A significant portion of the nanoplankton, in addition to the microzooplankton we studied, may also be susceptible to filtration damage and contribute to the apparent biomass and activity of picoplankton as seen by filter-fractionation (G. Fahnenstiel, G.L.E.R.L., Ann Arbor, MI, pers. comm.). Further, imperfections in the filters themselves (Stockner et al. 1990) may be a source of underestimation of biomass in smaller particle size-classes. The net effect of these problems is likely to be that nanoplankton and microplankton biomass and tracer uptake are underestimated by filter-fractionation, while dissolved and possibly picoplankton biomass and activity are overestimated. An extreme view would be that most of the filtrate P (about 30% of the total) is derived from cell damage and leaky filters. However, that a large component of particulate P and phosphate assimilation is by organisms other than nanoplankton is not an untenable proposition. The expectation that most of the biomass in an ecosystem is autotrophic, and most P uptake is by autotrophs, is fostered by a trophic-dynamic viewpoint not in tune with our current knowledge of the cycling at the base of the food chain. Although respiration cannot exceed photosynthesis without import of fixed C, secondary production can exceed primary production and bacterial uptake of DOC can be similar to primary production (e.g. Scavia 1988). Also, phototrophic picoplankton contribute a substantial fraction of phytoplankton biomass in many lakes, including Jacks Lake (Pick and Agbeti 1991). Therefore, it is possible that picoplankton do have a large requirement for P.

There are in situ estimates of nanoplankton grazing on picoplankton to support the rates we derived indirectly for our budget. Flagellated nanoplankton are widely believed to perform most of the grazing on picoplankton in marine plankton, and this may be often true of freshwater as well (Weisse 1990). Fig. 3C indicates that picoplankton are cleared at a rate of $38\%\cdot d^{-1}$, with only a modest $16\%\cdot d^{-1}$ resulting from the feeding of nanoplankton. In Lake Oglethorpe, Georgia, heterotrophic and mixotrophic flagellates performed 68–94% of bacterivory (Sanders et al. 1989), with their minimum contribution occurring in June. A large contribution to bacterivory by nanoplankton has been observed in other lakes as well (Bloem et al. 1989; Zinabu and Taylor 1990), although *Daphnia* performed most grazing in July in Upton Lake, New York (Pace et al. 1990). Equal or higher grazing rates by nanoplankton on picoplankton have been measured in coastal marine environments (Andersen and Sørensen 1986; Sherr et al.

1989) and in lakes (Zinabu and Taylor 1990; Bloem et al. 1989; Weisse 1990). Lower rates were measured on most dates by Sanders et al. (1989) in Lake Oglethorpe and by Simec et al. (1990) in a mesotrophic to eutrophic reservoir in Czechoslovakia.

In the epilimnion of Jacks Lake, heterotrophic flagellates were estimated as 1260 and 400·mL^{-1} on two occasions during our study. The biomass of these plus potentially mixotrophic forms was about 270 mg·m^{-3} or 10–34% of the phytoplankton biomass (F. Pick and M. Agbeti, University of Ottawa, Ottawa, Ont., pers. comm.). A similar abundance of heterotrophic flagellates in Lake Ontario (Pick and Caron 1987) was estimated to have a clearance rate for picoplankton of 36%·d^{-1} (Lean et al. 1987b).

An interesting feature of the budget model presented in Fig. 3C is that the sizes and turnover rates for P compartments are of the same order for picoplankton, nanoplankton, and zooplankton. The rapid turnover times for zooplankton P we found are, in fact, similar to those found in a laboratory study (Lehman and Naumoski 1985). They used comparable methods to show that turnover times for P in *Daphnia pulex* were 36–47.6 h when the *Daphnia* grazed on algae reared under low-P conditions. Using data for comparable 1500-μm *Daphnia* from Tables 4 and 5, we calculated a P turnover time of about 34 h. Lehman and Naumoski (1985) also obtained similar but higher values for P content of *Daphnia*, suggesting that their animals were in a better nutritional state. That is, they measured higher assimilation rates and P contents to estimate similar turnover times.

A problem with using these tracer techniques in lake water rather than laboratory cultures is that of uncertainty concerning isotopic equilibrium. If slower growing organisms, probably zooplankton, were not fully labelled during our incubations, then estimates of their P content and P release rate would be low. We only used ^{32}P to estimate P content for ciliates, the fastest growing of the zooplankton, and could compare these values with P/volume estimates from laboratory cultures (see Materials and Methods).

The problem of underlabelled P being released from large zooplankton is greater. Community estimates of zooplankton excretion have been performed for a suite of lakes using chemical methods to assay P release (Ejsmont-Karabin 1983). These estimates are high relative to our estimates for Jacks lake, but are for lakes of higher total P; extrapolating them using the equation accompanying fig. 2 in Ejsmont-Karabin (1983) produces an estimate three times higher than ours (2406 compared with 748 pM·h^{-1}). This partly reflects higher estimates of release per zooplankton. Our largest *Daphnia* (mean length 1572 μm) should weigh about 14.7 μg (using the same length–weight conversion as Ejsmont-Karabin, from Bottrell et al. 1976). Ejsmont-Karabin (1984) predicts an excretion rate of 117 pM·h^{-1} for cladocerans of this size, about twice as high as we observed (69 pM·h^{-1}, Table 4). The discrepancy between these estimates is probably too great to be from incomplete labelling of pools, but is within the range to be expected from differences in nutrition (Peters and Rigler 1973; Ejsmont-Karabin 1984; Lehman and Naumoski 1985). However, if our excretion rates are underestimated for larger animals, the net results for our budget model would be that the pico- and nanoplankton pools would suffer higher grazing and have shorter turnover times.

We conclude that by disregarding our postfiltration estimates of phosphate concentration in lake water, and by including recent advances in our understanding of trophic interactions at the base of pelagic food webs, observations concerning P cycling in the epilimnion of Jacks Lake can be accommodated into a plausible budget model. Remaining uncertainties include the size and dynamics of dissolved P compartments and whether the P model can be reconciled with a C budget.

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Influence of Photoperiod on the Number and Ultrastructure of Gill Chloride Cells of the Atlantic Salmon (*Salmo salar*) before and during Smoltification

Robert T. Lubin and A. W. Rourke

Department of Biological Sciences, University of Idaho, Moscow, ID 83483, USA

and Richard L. Saunders

Department of Fisheries and Oceans, Fisheries and Environmental Sciences, Biological Station, St. Andrews, N.B. E0G 2X0, Canada

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Atlantic salmon (*Salmo salar*) larger than 9 cm held under constant light since October had greater numbers of gill chloride cells in February compared with fish held under simulated natural photoperiod. By April, fish raised under simulated natural photoperiod were able to survive salinity tests, had a threefold increase in Na^+/K^+ ATPase activity, and had increased chloride cell numbers. These increases and salinity tolerance did not occur in fish raised under constant light. Only fish reared under constant light developed apical pits with large and numerous microvilli.

Chez des saumons de l'Atlantique (*Salmo salar*) de plus de 9 cm gardés sous un éclairage constant depuis le mois d'octobre, nous avons constaté en février que le nombre de cellules à chlorure branchiales était plus élevé que chez des poissons gardés dans une simulation de la photopériode naturelle. En avril, les poissons soumis à la photopériode naturelle simulée pouvaient survivre aux tests de salinité; de plus, chez ces poissons, l'activité Na^+/K^+ ATPase avait triplé et le nombe de cellules à chlorure avait augmenté. Il n'y a rien eu de comparable comme augmentation ou comme tolérance à la salinité chez les poissons gardés sous éclairage constant et c'est seulement chez ces derniers qu'on a observé des fosses apicales à microvillosités nombreuses et développées.

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Branchial chloride cells in gills of teleosts are generally large, columnar in shape with single nuclei, numerous mitochondria, an extensive tubular membrane system, and numerous vesicles in the apical cytoplasm (Karnaky 1986; Pisam et al. 1988). These cells may have a role in ion tansport in both freshwater- and saltwater-adapted teleosts.

Foskett et al. (1983) have suggested that the development of the ion transport mechanism in euryhaline teleosts involves activation and proliferation of immature chloride cells followed by differentiation of these cells. Such differentiation is characterized by increased activity of enzymes such as the Na^+/K^+ ATPase pump and mitochondrial succinate dehydrogenase (Shirai and Utida 1970; Karnaky 1986; Thomson and Sargent 1977). Studies with anadromous Atlantic salmon (*Salmo salar*) have shown increases in chloride cell number and size when the salmon are transferred to salt water (Burton and Idler 1984; Langdon and Thorpe 1984). Furthermore, differentiation has been shown to occur as exposure to salt water increases activities of the Na^+/K^+ pump and mitochondrial succinic acid dehydrogenase (Langdon and Thorpe 1984, 1985).

Saunders et al. (1985) and McCormick et al. (1987) have shown that Atlantic salmon raised under constant light do not develop smolt characteristics, as they do not show spring increases in gill Na^+/K^+ ATPase activity, and such fish do not pass a saltwater challenge. This fact could indicate that these fish either have few chloride cells present which may or may not be highly differentiated or that such fish have undergone chloride cell proliferation without any accompanying differ-

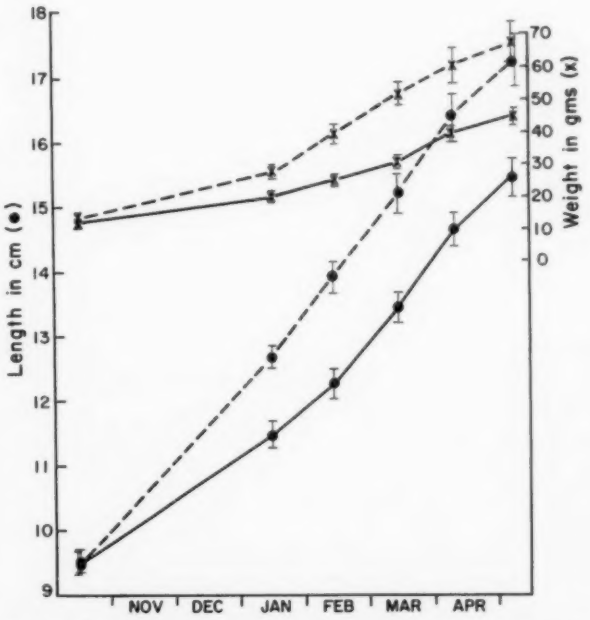

FIG. 1. Growth in length and weight of Atlantic salmon raised under two photoperiods: CL (broken lines) and SNP (solid lines). Data are from 1988 fish.

TABLE 1. Area and number of chloride cells in gills of Atlantic salmon raised since October under simulated natural photoperiod (SNP) or constant light (CL).

	Area (μm^2)[a]		Number per interlamellar area[b]	
	SNP	CL	SNP	CL
February	130 ± 35 a	118 ± 18 a	2.1 ± 0.47 a	4.1 ± 0.87 b
March	100 ± 20 a	94 ± 21 a	3.1 ± 0.67 a	3.7 ± 0.82 b
April	105 ± 24 a	96 ± 19 a	3.8 ± 0.99 b	3.9 ± 0.99 b
May	110 ± 28 a	100 ± 22 a	4.0 ± 0.78 b	4.1 ± 0.56 b

[a]Values followed by the same letters are not significantly different at the 0.05 level as judged by Duncan's multiple range test.

[b]Values followed by different letters are significantly different as judged by a Kruskal–Wallis one-way analysis of variance. Only values from transmission electron micrographs are reported; values from ZnI_2-stained gills showed no significant differences with transmission electron micrograph values.

entiation. Constant light could influence chloride cell proliferation and/or differentiation. The aim of the present ultrastructural study was to compare the influence of constant light and simulated natural photoperiod on the development of the gill chloride cells in Atlantic salmon.

Materials and Methods

Saint John stock Atlantic salmon from the Mactaquac Federal Fish Hatchery near Fredericton, New Brunswick, Canada, were used in this study. The fish were received as swim-up fry at the St. Andrews Biological Station, graded in late October, and parr large enough (>9 cm) to become smolts the following spring were retained and distributed into four 1-m^2 fiberglass tanks. The tanks were supplied with dechlorinated freshwater at 12°C with flow rates of 12 ± 1 L·min^{-1} and illuminated using a pair of 40-W daylight fluorescent lamps suspended 1 m above the water surface. Photoperiods were simulated natural (SNP) for latitude 45°N in two of the tanks and constant light (CL) in the remaining two tanks. Fish were raised both in 1988 and 1989. The fish were fed commercial salmon food (Ewos or Silver Cup) at satiation rates. Food was placed daily in automatic feeders set to dispense pellets to all groups during the light hours of the SNP group.

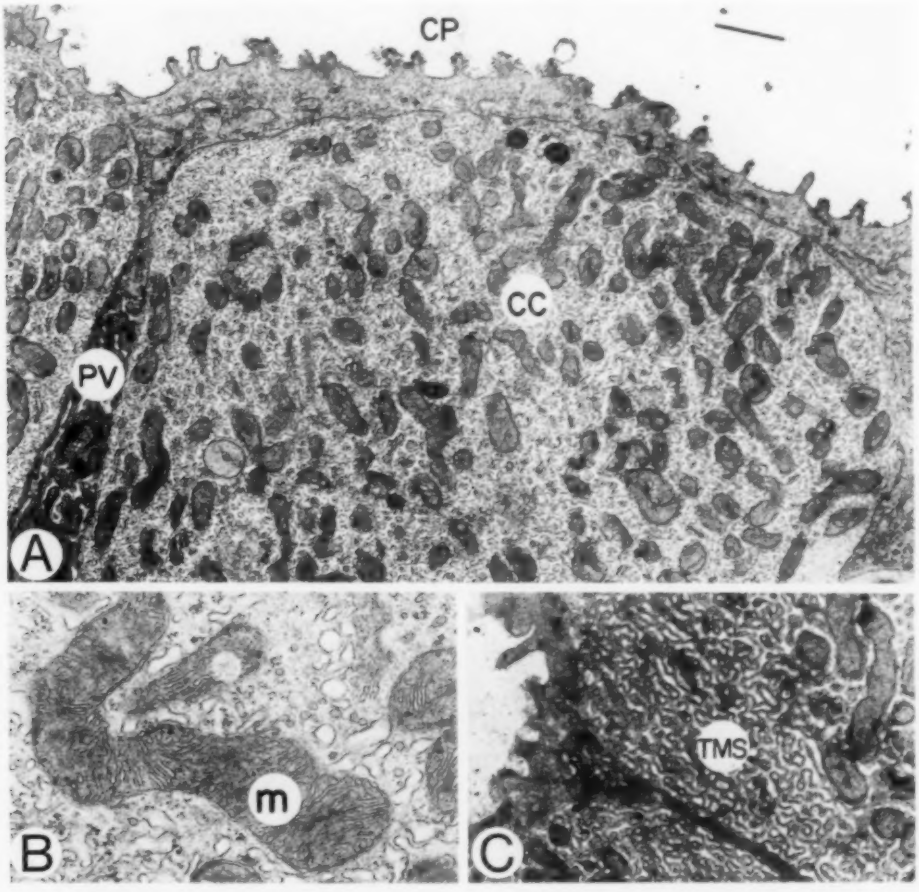

FIG. 2. (A) Typical electron micrograph of a chloride cell (CC) with an associated pavement cell (PV) having cytoplasmic projections (CP) (8500×). (B) Elongate mitochondria (m) (25 000×) and (C) tubular membrane systems (TMS) (12 000×) were characteristic of all fish at all times. All figures are for February SNP fish. Similar cells and organelles were seen in micrographs of samples from CL fish.

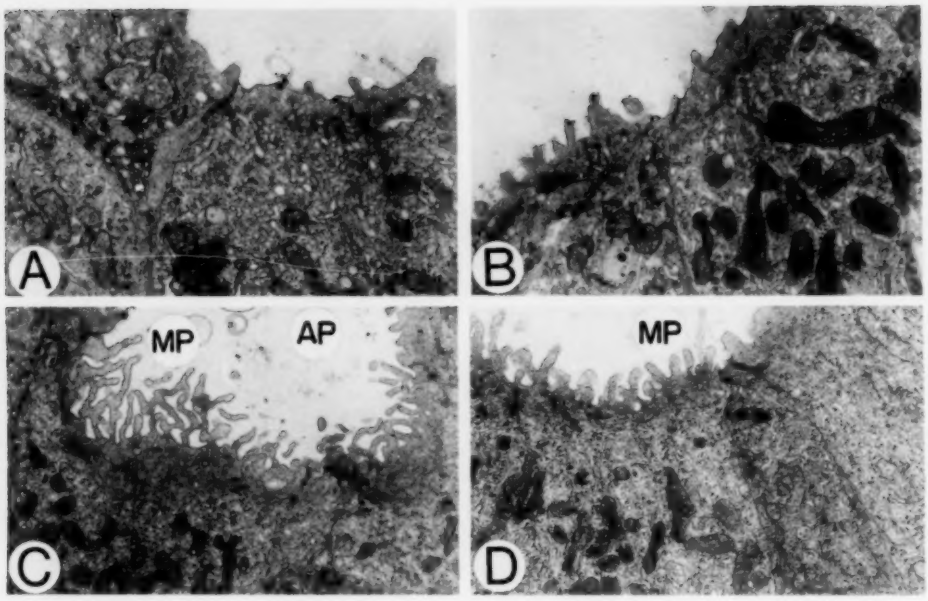

FIG. 3. Exposed chloride cells with microvilli-like projections (MP) from March fish held under (A) SNP and (B) CL (both lacking an apical pit) and April fish held under (C) CL and (D) SNP (10 000×). Only April CL fish showed apical pits (AP).

Salinity tolerance tests were conducted on 1988 fish twice during the experiment. The fish were challenged for 96 h with water of 37.5 ‰ salinity, prepared by adding Instant Ocean salt to laboratory salt water. Ten fish were taken randomly from each photoperiod regime (half from each replicate) and transferred directly to salinity tolerance tanks in which temperature was held at 12°C. The static water was aerated continuously to maintain oxygen levels >80‰ of air saturation. Fish were not fed 1 d prior to or during the tests (Saunders et al. 1985). Assays for gill Na^+/K^+ ATPase were run on samples from February through May using the procedures described by McCormick et al. (1987). Protein content of the enzyme preparations was determined by the method of Bradford (1976) with bovine serum albumin (BSA) used as standard.

Once a month the weight and fork lengths of all fish were determined. Every 2 wk, three individual fish from each photoperiod were examined for coloration and then killed by a sharp blow to the head. The second most anterior gill arch on the left side of three fish from each photoperiod treatment was immediately fixed in a 2.5% glutaraldehyde for 3 h, transferred to 0.1 M cacodylate buffer, postfixed in a 1% osmium tetroxide, dehydrated in ethanol, and embedded in Spurr's low-viscosity embedding medium. Silver–gold (60–90 μm) thin sections were cut using an LKB Ultrotome III ultramicrotome with a Diatome diamond knife, stained with uranyl acetate and lead citrate (Reynolds 1963), and examined with a Zeiss EM 10 transmission electron microscope. In 1989, samples were prepared in this fashion, and an additional four samples per photoperiod group were prepared from the same gills used for electron microscopy for staining with ZnI_2 to differentially stain chloride cells (Madsen 1990). The total sample size for electron microscopy was seven fish per group per time point with three samples prepared for each point in 1986 and four in 1989.

The interlamellar region, defined as the area between two secondary lamellae on a filament, was utilized for gill chloride cell examination. Electron micrographs were normally taken at five different magnifications (3125 to 40 000×) (Dupont EM-7 film) within randomly located interlamellar regions on thin sections.

Chloride cell densities were determined by counting the number of chloride cell nuclei appearing per rectangular area on five interlamellar regions per fish using the 3125× micrographs in both 1986 and 1989. A total of seven fish per photoperiod per date were examined. In 1989, additional counts of four interlamellar areas in ZnI_2-treated samples were made at 400× with a light microscope. Choride cell size was estimated by measuring length from basal to apical surfaces through the nucleus in five randomly selected cells found on the interlamellar region for each photoperiod at each sampling date using the 3125× micrographs.

Results

The time course of acquisition of smolt status was assessed by observation of coloration, Na^+/K^+ ATPase activities, and salinity tolerance tests. Salinity tolerance tests (96 h) in mid-April and early May resulted in 100% survival of fish raised in SNP and 0% survival of CL fish. This was the case even though CL fish displayed higher growth rates and reached larger size than SNP fish (Fig. 1). Gill Na^+/K^+ ATPase increased threefold from an average of 3.1 μmol Pi·mg protein^{-1}·h^{-1} in February to 9.9 in April in fish raised under SNP, but remained unchanged in fish raised under CL (3.6 μmol Pi·mg protein^{-1}·h^{-1} in February to 3.7 in April). The coloration of the caudal and pectoral fin tips changed from light to dark in the fish raised under SNP, but remained relatively unchanged in the fish raised under CL.

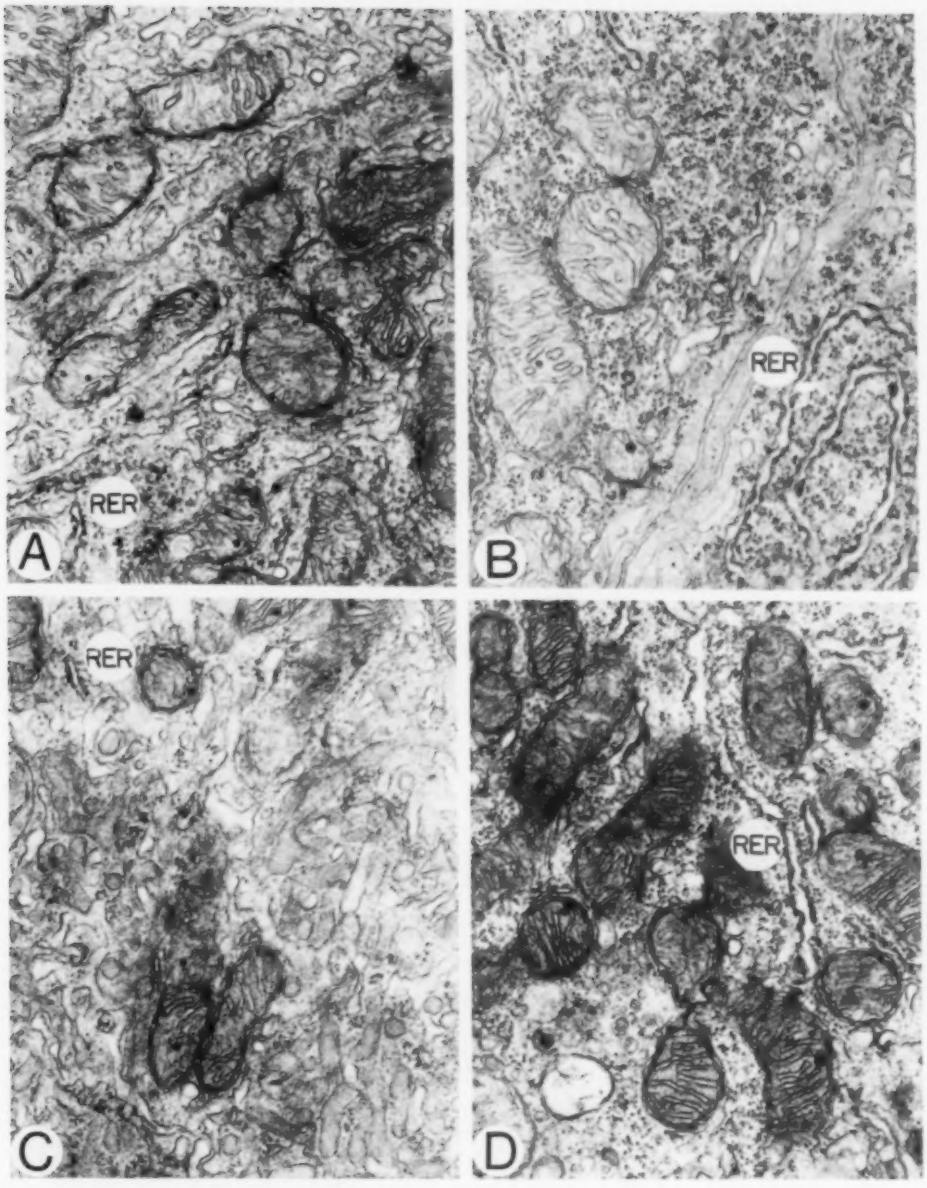

FIG. 4. Free ribosomes and rough endoplasmic reticulum (RER) in chloride cells of fish raised under (A and B) SNP and (C and D) CL in March (Fig. 4A and 4C), April (Fig. 4B), and May (Fig. 4D) (32 000×).

The number of chloride cells in fish raised under SNP increased significantly during the study period (Table 1). The number of chloride cells in fish raised under CL was significantly higher than in fish raised under SNP early in the study period. The size of chloride cells was not different between photoperiod groups and did not show significant changes with time.

Transmission electron micrographs revealed cytoplasmic projections of pavement cells overlying most chloride cells in all sampled groups (Fig. 2) at all time periods. Mitochondria were found distributed throughout chloride cells and were both elongate and spherical with well-developed cristae (Fig. 2B). The smooth membrane system, which includes vesicles found in the apical region of the chloride cells and a basolateral tubular membrane system, was extensive in both photoperiod groups throughout the experiment (Fig. 2C). Apical vesicles and the tubular membrane system were extensive from February through mid-May.

Contrasting microanatomical features developed in the fish raised under the two photopriods as gill ATPase activities increased. Apical pits were not present in exposed chloride cells in either photoperiod group during February or March (Fig. 3A and 3B) but did not develop in surface exposed chloride cells in April fish raised under CL (Fig. 3C). Such structures were

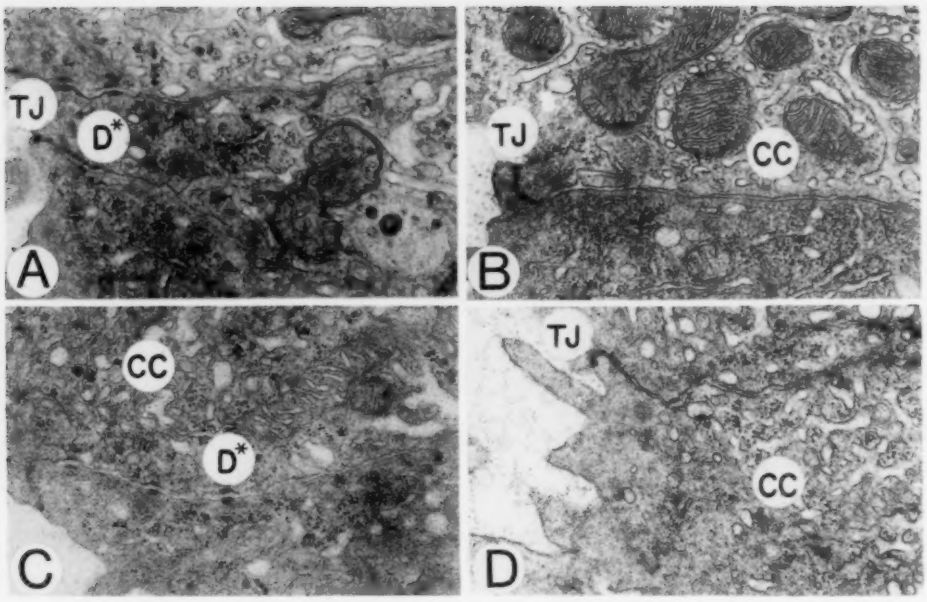

FIG. 5. Long junctions between chloride cells (CC) and adjacent cells with typical desmosomes (D*) and tight junctions (TJ) in February fish raised under (A) SNP and (B) CL and in May fish raised under (C) SNP and (D) CL (26 000×).

not observed in fish raised under SNP (Fig. 3D). Microvilli-like projections protruded from both pavement cells and exposed chloride cells directly into the external milieu during the experiment in both photoperiod groups. However, chloride cell microvilli-like projections were more numerous and longer in the April and May fish raised under CL than any other group sampled (Fig. 3). Free ribosomes and rough endoplasmic reticulum were found in both photoperiod treatment groups at all times. (Fig. 4). Chloride cells in both groups of fish were firmly bound by long junctions to pavement cells and/or other chloride cells. It was common for limiting membranes in samples from February to May to form typical desmosomes just below plasma membranes and for the juxtaposed membranes between the desmosomes and the external milieu to form tight junctions (Fig. 5). Leaky junctions and accessory chloride cells like those in saltwater-adapted eels (Laurent and Dunel 1980) were not observed.

Discussion

The present analysis of the effects of photoperiod on Atlantic salmon indicates that fish raised under CL do not develop a preadaptation to seawater whereas those under SNP do. Fish raised under CL are unable to pass a salinity tolerance test, their Na^+/K^+ ATPase levels remain unchanged during April, and chloride cell numbers are the same in February as in May (McCormick et al. 1987). Fish raised under SNP do pass salt challenges, their Na^+/K^+ ATPase activity increases in April, and chloride cell numbers increase significantly from February to May, as also determined by Burton and Idler (1984) and Langdon and Thorpe (1985).

In February the CL fish have chloride cell numbers equaling those of smolts, May fish raised under SNP (Table 1). However, the chloride cells in fish raised under CL do not continue to proliferate during March, April, and May and do not display the same ultrastructural changes (Fig. 3) seen in fish raised under SNP. The initially large numbers of chloride cells of CL fish in February is probably due to the early stimulatory effects received from transfer to CL conditions. This number and the number of chloride cells present per interlamellar area in May in SNP fish are comparable with that characteristic of sea trout parr (*Salmo trutta trutta*) stimulated with cortisol and growth hormone (Madsen 1990). The lack of further proliferation of chloride cells in Atlantic salmon raised under CL may be due to the effect of their being exposed to one large increase in day length and not steadily increasing day lengths. Komourdjian et al. (1976) reported early development of smolt chaacteristics in juvenile Atlantic salmon subjected to a reciprocal photoperiod (increased from SNP in December to 16 h light : 8 h dark and decreasing to 8 h light : 16 h dark in June). The present report indicates that day length appears to affect chloride cell numbers in both SNP and CL fish, and there appears to be a relationship between the extent of proliferation and the extent of the observed increase in day length. Further, it appears that differentiation is stimulated by gradually increasing day lengths and not one large increase in day length to CL conditions.

The apical pits which developed in April in fish raised under CL are not typical of transmission electron micrographs of freshwater Alantic salmon (Lubin et al. 1989), the Japanese eel (*Anguilla japonica*) (Shirai and Utida 1970), the sole (Dunel-Erb and Laurent 1980), and the killifish (*Fundulus heteroclitus*) (Hossler et al. 1985). The formation of the pits in fish raised under CL may represent an attempt by these fish to increase the surface area of the chloride cells exposed to the external milieu to faciliate ion uptake in freshwater like that reported in Baltic Sea Atlantic salmon (Chernitsky 1980). This explanation might also apply to the increase in microvilli observed in April and May in fish raised under CL.

The data from this study do not indicate any statistically significant increase in the size of the chloride cells in either photoperiod group (Table 1). The data are consistent with those reported by Pisam et al. (1988) and concur with Lubin et al. (1989) and with Birt and Green's (1986) study of juvenile anadromous and nonanadromous Atlantic salmon but not with those of Langdon and Thorpe (1985). The reason for the differing results is not clear, but might relate to strain differences or differences in methods. Langdon and Thorpe (1985) measured diameters of cells, while the present report, the work of Birt and Green (1986), and Lubin et al. (1989) measured length through the nucleus.

Acknowledgements

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Distribution and Phenotypic Correlates of Variation at Enzyme Coding Loci in Rainbow Trout (*Oncorhynchus mykiss*) from the Lower Laurentian Great Lakes

Moira M. Ferguson

Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1, Canada

and Peter E. Ihssen

Research Section, Fisheries Branch, Ontario Ministry of Natural Resources, Maple, Ont. L6A 1S9, Canada

Ferguson, M. M., and P. E. Ihssen. 1991. Distribution and phenotypic correlates of variation at enzyme coding loci in rainbow trout (*Oncorhynchus mykiss*) from the lower Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. 48: 1308–1315.

The distribution of variation at 37 enzyme coding loci was determined in six samples of rainbow trout (*Oncorhynchus mykiss*) from hatchery and natural sources. Significant heterogeneity in allele frequencies was detected among samples of Lower Laurentian Great Lakes rainbow trout. Geographical proximity was a poor predictor of genetic similarity among samples. The adaptive significance of two loci was examined in 16 experimental families. Rainbow trout homozygous for an allele at a phosphoglucomutase structural gene (*Pgm1-s*), which results in no detectable activity of phosphoglucomutase (PGM1) in all tissues where this locus is normally expressed (e.g. muscle), were larger than those heterozygous or homozygous for the active allele. However, no significant differences in asymmetry of four bilateral meristic traits were detected between fish with different PGM1 phenotypes. Rainbow trout with PGM1 in liver because of a regulatory gene, *Pgm1-t(b)*, were marginally larger than those without the allele. These data suggest a correlation between biochemical genetic variation and phenotypic characteristics associated with fitness.

Nous avons examiné la répartition de la variation à 37 loci responsables du codage des enzymes dans six échantillons de truite arc-en-ciel (*Oncorhynchus mykiss*) provenant de pisciculture et prises dans le milieu naturel. On a détecté une nette hétérogénéité dans la fréquence des allèles entre les échantillons de truite arc-en-ciel venant des Grands Lacs inférieurs. La proximité géographique n'a été qu'un médiocre prédicteur de la similarité génétique entre les échantillons. L'importance des deux loci sur le plan de l'adaptation a été examinée dans 16 familles soumises à l'expérience. Les truites arc-en-ciel homozygotes pour un allèle correspondant à un gène structural de la phosphoglucomutase (*Pgm1-s*), qui ne produit aucune activité détectable de la phosphoglucomutase (PGM1) dans tous les tissus où ce locus s'exprime normalement (p. ex. les muscles), étaient de plus grande taille que les poissons hétérozygotes ou homozygotes pour l'allèle actif. On n'a toutefois relevé aucune différence significative dans l'assymétrie de quatre traits méristiques bilatéraux entre des poissons présentant des phénotypes différents en ce qui concerne la PGM1. Les truites arc-en-ciel présentant la PGM1 dans le foie à cause d'un gène de régulation, *Pgm1-t(b)*, étaient légèrement plus grosses que celles qui ne possédaient pas l'allèle. Ces données semblent indiquer une corrélation entre la variation génétique au niveau biochimique et les caractéristiques phénotypiques liées au bon état général.

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The significance of variation at enzyme coding loci remains controversial (Ryman and Utter 1987; Zouros and Foltz 1987). Alleles that code for differences in enzyme activity might have greater adaptive significance than those that lead to differences in electrophoretic mobility. For example, rainbow trout (*Oncorhynchus mykiss*) from Montana with increased phosphoglucomutase enzyme (PGM) activity in liver because of a regulatory gene *Pgm1-t(b)* (Allendorf et al. 1982) develop more quickly, are larger as juveniles, and show higher levels of agonistic behaviour than their full-sibs with reduced enzyme activity (Allendorf et al. 1983; Ferguson and Danzmann 1985; Danzmann et al. 1986; Ferguson et al. 1988a).

Fish with alleles that produce no detectable enzyme product (i.e. null alleles) might be expected to have reduced fitness depending upon the importance of the enzyme function in metabolism. Several null alleles have been reported in salmonid fishes (Klar and Stalnaker 1979; Stoneking et al. 1981; Allendorf et al. 1984) but few studies have provided direct evidence of null alleles on fitness. Rainbow trout with an *Ldh3* null allele develop more slowly (Ferguson et al. 1988b) and show reduced developmental stability as measured by fluctuating asymmetry of bilateral meristic characters than fish with normal activity (Knudsen et al. 1984). The distribution and phenotypic effects of such enzyme variation have not been established in stocks of rainbow trout in the lower Great Lakes. Krueger and May (1987) examined the population genetic structure of rainbow trout from Lake Superior tributaries but did not analyze alleles characterized by differences in enzyme activity.

The first objective of the present study was to determine the distribution and frequency of variation at enzyme coding loci

of potential significance in natural and hatchery populations of rainbow trout from the lower Laurentian Great Lakes. Specifically, samples were screened for null alleles and the *Pgm1-t(b)* allele. Once specific polymorphisms were identified, the second objective was to determine if the differences in enzyme activity produced by these genes were associated with important phenotypic effects such as size and developmental stability. Fluctuating asymmetry of bilateral meristic characters is commonly used as a measure of developmental stability (Leary et al. 1985). Asymmetry or differences between counts of characters on the left and right sides of the same organism is thought to reflect accidents during development (at the time the characters are determined). Therefore, developmental stability as reflected by reduced fluctuating asymmetry of bilateral meristic traits is positively associated with fitness (Leary et al. 1985). Thus, fish with reduced asymmetry were expected to have greater fitness than those with higher asymmetry.

Materials and Methods

Allozyme Survey

Rainbow trout from tributaries of Georgian Bay, Lake Huron, Lake Erie, and Lake Ontario or their progeny as well as three Ontario Ministry of Natural Resources (OMNR) broodstocks from the Normandale Fish Culture Station (FCS) (Table 1; Fig. 1) were screened with starch gel electrophoresis according to the methods of Allendorf et al. (1977). The following enzymes encoding 37 loci were screened: *N*-acetyl-beta glucosaminidase (bGLUA; EC 3.2.1.30), adenylate kinase (ADK; EC 2.7.4.3), alcohol dehydrogenase (ADH; EC 1.1.1.1), creatine kinase (CK; EC 2.7.3.2), dipeptidase (glycyl-leucine) (PEPA; EC 3.4.13.11), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), glycerol-3-phosphate dehydrogenase (G3PDH; EC 1.1.1.8), L-iditol dehydrogenase (IDDH; EC 1.1.1.14), isocitrate dehydrogenase (IDH; EC 1.1.1.42), lactate dehydrogenase (LDH; EC 1.1.1.27), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.37), phosphoglucomutase (PGM; EC 5.4.2.2), phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44), superoxide dismutase (SOD; EC 1.15.1.1), tripeptide aminopeptidase (PEPB; EC 3.4.11.4), and xanthine dehydrogenase (XDH; EC 1.1.1.20). The nomenclature described by Allendorf et al. (1983) was used where, for example, IDH2 designates the electrophoretically detectable enzyme produced by the structural locus *Idh2*. Electrophoretic mobilities of the protein products were based on previous designations (Ferguson et al. 1985). The frequencies of null alleles were estimated by taking the square root of the frequency of null homozygotes assuming Hardy–Weinberg proportions. For each pair of duplicated loci, individuals with more than one electromorph were designated heterozygotes and allele frequencies were estimated by allele counting. Average heterozygosites were expressed as expected values from Hardy–Weinberg expectations except for the duplicated loci where observed values were used. Variation at the duplicated loci was not examined for conformation to Hardy–Weinberg expectations.

Phenotypic Effects

The phenotypic effects of variation at two loci, *Pgm1-s* and *Pgm1-t*, were determined with two sets of experimental matings. Thirty-six males from the pooled progeny cross from the Saugeen River produced in April 1987 became sexually mature in April 1989. Six of these had liver PGM1 (*Pgm1-t(a/b)* genotypes) and each was crossed with two Saugeen females without liver PGM1 (*Pgm1-t(a/a)* genotypes) collected from the river on April 28, 1989. This resulted in 12 half-sib families each segregating 1:1 for the presence and absence of liver PGM1; the male parents must have been *Pgm1-t(a/b)*. Genetic crosses have shown that *Pgm1-t(b)* is inherited in a simple Mendelian fashion. (Allendorf et al. 1982). All fish were homozygous for the active allele at *Pgm1-s*. Each family was reared for about

TABLE 1. Summary of six samples of rainbow trout electrophoretically analyzed for variation at 37 enzyme loci.

Sample	Type of sample
Bothwell (BH)	OMNR hatchery stock, collected from Normandale FCS, September 1987 Parents of sampled fish collected from Bothwell Creek, Georgian Bay
Ganaraska (GH)	OMNR hatchery stock, collected from Normandale FCS, September 1987 Parents of sampled fish collected from Ganaraska River, Port Hope, Ontario
Nottawasaga (NH)	OMNR hatchery stock, collected from Normandale FCS, September 1987 Parents collected from Nottawasaga River, Ontario
Ganaraska River (GR)	Progeny produced from wild gamete collection on April 14–15, 1987, at Port Hope fishway (Lake Ontario); pooled mating (24♀ × 27♂)
Saugeen River (SR)	Progeny produced from wild gamete collection on April 10, 1987, at Denny's Dam fishway (Lake Huron); pooled mating (8♀ × 18♂)
Youngs Creek (YC)	Progeny produced from wild gamete collection on April 21, 1987 (Lake Erie); pooled mating (8♀ × 14♂)

NOTE: OMNR = Ontario Ministry of Natural Resources; N = 40 per sample.

FIG. 1. Origin of rainbow trout from the lower Laurentian Great Lakes. BC = Bothwell Creek, GR = Ganaraska River, NR = Nottawasaga River, SR = Saugeen River, YC = Youngs Creek.

4 mo after fertilization. A minimum of 35 fish was measured (fork lengths to the nearest millimetre) and their PGM1 phenotypes in liver were determined by cellulose acetate electrophoresis with a Tris–glycine buffer (Hebert and Beaton 1989).

As part of another experiment, 92 families were produced with Ganaraska rainbow trout collected from the Normandale FCS in March 1988. Prior to yolk sac resorption, a subsample of 12 progeny from each family was screened with starch gel electrophoresis for polymorphism at *Pgm1-s* in muscle. Four families were segregating for a null allele (*n*) at *Pgm1-s*. All parents must have had *Pgm1-s(100/n)* genotypes because all had some PGM1 in muscle and null homozygotes were observed in the progeny. All progeny had *Pgm1-t(a/a)* genotypes. At about 10 mo after fertilization, all progeny were measured (fork length to the nearest millimetre) and their muscle PGM1 phenotypes determined (active or null). The counts of the following characters on both the left and right sides of each fish were determined according to the methods of Hubbs and Lagler (1958): rays in the pectoral fin, rays in the pelvic fin, gill rakers on the upper part of the first branchial arch, and gill rakers on the lower branchial arch.

Results and Discussion

Genetic Differentiation

Thirteen of the 33 loci were polymorphic in at least one sample of rainbow trout (Table 2). Average heterozygosities ranged from 0.071 to 0.089 based upon 33 loci. All samples conformed to Hardy–Weinberg expectations except in two cases (*Idh2* in SR and *Pgm2* in NH) as indicated by log-likelihood *G*-tests (Sokal and Rohlf 1981). This is less than would be expected by chance alone and therefore likely of limited biological significance.

The six samples showed significant heterogeneity in allele frequencies at each of the six most polymorphic loci as indicated by contingency *G*-tests (Table 3). Variant alleles at *Mdh3,4* and *Idh3,4* were combined into one class. Nei's (1978) genetic distances (*D*) based upon the full set of 33 loci ranged from <0.001 to 0.013 (Table 4). The dendrogram constructed from these values (UPGMA; Sneath and Sokal 1973) was composed of five branches (Fig. 2). A hierarchical series of *G*-tests based upon numbers of alleles at the six most polymorphic loci were used to determine which of the branching points in the dendrogram were significant. For example, at node 2, the allele counts in SR were compared with the combined counts of GR, GH, YC, and NH. The numbers of significant loci at each branching point (largest to smallest) were 6, 2, 2, 1, and 0 (Table 3). Because the *G*-test is additive (Sokal and Rohlf 1981), the *G*-values from each locus were combined to give an overall statistic for the banching point. The combined analysis of loci indicated that all branches except the last were significant.

The genetic relatedness of the six samples of rainbow trout from the lower Laurentian Great Lakes, as indicated by the dendrogram of genetic distances (Fig. 2), does not correspond to the geographic proximity of the source samples (Fig. 1). For example, NH and YC showed the least divergence, yet NH was derived recently from a tributary of Georgian Bay and YC from fish spawning in a tributary of Lake Erie. This pattern stands in contrast with that reported by Krueger and May (1987) in that groups of collections from Lake Superior tributaries identified by cluster analysis of genetic distances usually contained samples that were geographically proximate.

The apparent difference in population structuring could be explained by differences in stocking histories between the populations in Lake Superior and the southern lakes. Krueger and May (1987) have suggested that the level of differentiation among rainbow trout populations is a function of time that populations have had to diverge; longer times would result in greater differentiation. For instance, trout from around Lake

TABLE 2. Allozyme variation in six samples of rainbow trout from the Lower Laurentian Great Lakes. Key to sample abbreviations is given in Table 1.

		Hatchery			Wild gamete progeny		
Locus	Allele	BH	GH	NH	GR	SR	YC
Ckl	*100*	1.000	0.975	0.988	1.000	1.000	1.000
	76	—	0.025	0.012	—	—	—
PepAl	*100*	1.000	0.975	1.000	0.950	1.000	1.000
	120	—	0.025	—	0.050	—	—
G3pdh	*100*	1.000	0.937	0.950	0.937	1.000	1.000
	140	—	0.063	0.050	0.063	—	—
bGlua	*100*	0.875	0.725	0.711	0.936	0.743	0.729
	75	0.125	0.275	0.289	0.064	0.257	0.271
Iddh	*100*	0.967	1.000	1.000	1.000	0.971	0.987
	60	0.063	—	—	—	0.029	0.013
Idh2	*100*	0.850	0.825	0.750	0.887	0.592	0.700
	140	0.150	0.175	0.250	0.113	0.408	0.300
Idh3,4	*100*	0.719	0.859	0.851	0.855	0.800	0.868
	114	0.093	0.032	0.018	0.019	0.043	0.019
	71	0.130	0.045	0.056	0.088	0.093	—
	40	0.175	0.064	0.075	0.038	0.064	0.113
Ldh4	*100*	0.962	1.000	0.975	1.000	1.000	0.950
	76	0.038	—	0.025	—	—	0.050
Mdh3,4	*100*	0.925	0.859	0.825	0.863	0.875	0.818
	125	—	0.026	—	0.018	—	0.019
	83	0.075	0.115	0.175	0.199	0.125	0.163
Me3	*100*	1.000	0.975	1.000	0.975	0.986	1.000
	75	—	0.025	—	0.025	0.014	—
Pgm1-s	*100*	1.000	1.000	1.000	0.841	1.000	1.000
	Null	—	—	—	0.158	—	—
Pgm1-t	*a*	1.000	0.987	1.000	1.000	0.900	1.000
	b	—	0.013	—	—	0.100	—
Pgm2	*100*	0.475	0.875	0.718	0.862	0.842	0.700
	90	0.525	0.125	0.282	0.138	0.158	0.300
Sod	*100*	0.900	0.847	0.731	0.725	0.471	0.706
	152	0.100	0.153	0.269	0.275	0.529	0.294
Het[a] (33 loci)		0.071	0.071	0.086	0.076	0.089	0.084

[a]Calculated from expected heterozygosities except for *Mdh3,4* and *Idh3,4* where observed values were used.

Superior have reduced differentiation relative to samples from Pacific streams (e.g. Parkinson 1984), possibly as a function of their recent introduction into the Laurentian Great Lakes. The time of first introduction of rainbow trout into the southern and northern Great Lakes is similar (MacCrimmon 1971) so that differences in population differentiation are not a simple function of potential divergence time. More recent stocking between local populations in the southern lakes could account for less population differentiation.

Genetic Diversity of Hatchery Fish

We compared the amounts of heterozygosity in the OMNR brookstocks, in other hatchery stocks (Ferguson et al. 1985), and naturalized populations (present study; Krueger and May 1987) to determine if changes in heterozygosity have occurred during the development of the stocks. The expected heterozygosities were calculated for the 14 loci in common to these three studies including the duplicated loci. It would have been preferable to use observed values for the duplicated loci but it was not possible to derive these from the allele frequencies reported by Krueger and May (1987). The three OMNR brood stocks had marginally lower amounts of expected enzyme heterozygosity on average (0.119) than the three progeny groups produced from wild-caught fish examined in the present study (0.127) and the samples of Krueger and May (1987) (0.141) based on the common set of 14 loci. The average heterozygosities of the OMNR stocks were, however, higher than those in some U.S. Fish and Wildlife hatchery stocks of rainbow trout that had been maintained in culture for many years (Danzmann et al. 1986).

The allele frequencies of GH were compared with GR with a contingency *G*-test of allele counts to determine if the hatchery fish have diverged genetically from the natural population. Significant differences were detected at one of six loci, only. Thus, the controlled breeding program for the OMNR broodstocks (Ferguson et al. 1991) has apparently been successful in maintaining genetic variation, as no major differences in allele frequencies (one of six loci examined) and heterozygosity (0.071 versus 0.076) were detected between GH and GR. Ferguson et al. (1991) performed a more extensive comparison between the original rainbow trout collected from the Ganaraska River used to found the hatchery stock (N = 876) in 1983, 1984, and 1985 and three descendant lines at Normandale FCS assayed in spring 1990. Despite the large numbers of fish electrophoretically analyzed, no significant differences in either heterozygosity or allele frequencies were detected between river and descendant hatchery fish.

The lack of detectable genetic differences between wild and hachery stocks of Ganaraska River rainbow trout is consistent with the goal of many fish culture programs where the objective is to maintain genetic variation in the hatchery stocks and minimize changes in allele frequencies (Allendorf and Ryman 1987; Quattro and Vrijenhoek 1989). This is because electrophoretically detectable variation is positively correlated with characters related to fitness. For example, hatchery stocks of rainbow trout with higher heterozygosities had faster developmental rates, as measured by hatching time, and a tendency to have larger eggs than those with lower heterozygosity (Danzmann et al. 1989). Furthermore, rainbow trout that survived an outbreak of bacterial gill disease were significantly more heterozygous than those that died (Ferguson and Drahushchak 1990).

Polymorphism at *Pgm1*

Variation at two loci differentially affects the amount of PGM1 enzyme in Ontario rainbow trout (Fig. 3). Most rainbow trout have PGM1 in all tissues except liver and kidney. Variation at the regulatory locus, *Pgm1-t*, controls the expression of *Pgm1-s* in liver (Allendorf et al. 1982). Fish with the *Pgm1-t(b)* allele (homozygous or heterozygous) have liver PGM1 whereas those homozygous for the *Pgm1-t(a)* allele do not (Fig. 3B). The *Pgm1-t* locus does not affect muscle PGM1, so all fish, regardless of *Pgm1-t* genotype, have muscle PGM1 activity (Fig. 3A). The *Pgm1-t(b)* allele was detected in progeny produced from gametes collected from Saugeen rainbow trout (Table 2). It was also observed in a single fish from the Ganaraska broodstock.

A null allele (*n*) was detected at the *Pgm1-s* structural gene. Homozygotes for the null allele (*Pgm1-s(n/n)* genotypes) do not have PGM1 enzyme in any tissue where *Pgm1-s* is normally

TABLE 3. Comparisons of genetic differences among six samples of rainbow trout from the lower Laurentian Great Lakes and the groupings in the dendrogram presented in Fig. 2. Values are *G*-statistics (df) and probability values from contingency tests of allele counts by locus.

Grouping	Locus						
	bGlua	*Pgm2*	*Idh2*	*Idh3,4*	*Mdh3,4*	*Sod*	Total
Separate	23.79 (5)	46.63 (5)	26.07 (5)	17.03 (5)	14.32 (5)	41.29 (5)	169.12 (30)
samples	<0.001	<0.001	<0.001	0.004	0.014	<0.001	<0.001
Node 1	4.83 (1)	32.94 (1)	3.85 (1)	13.92 (1)	10.90 (1)	15.85 (1)	82.30 (6)
(D=0.007)	0.028	<0.001	0.050	<0.001	<0.001	<0.001	<0.001
Node 2	0.35 (1)	1.16 (1)	11.99 (1)	2.87 (1)	1.23 (1)	19.95 (1)	37.51 (6)
(D=0.0046)	0.556	0.291	0.001	0.090	0.267	<0.001	<0.001
Node 3	18.54 (1)	3.69 (1)	6.69 (1)	0.01 (1)	0.85 (1)	0.40 (1)	30.19 (6)
(D=0.003)	<0.001	0.055	0.010	0.925	0.355	0.527	<0.001
Node 4	0.01 (1)	8.82 (1)	3.03 (1)	< 0.01 (1)	1.24 (1)	4.96 (1)	18.06 (6)
(D=0.0012)	0.936	0.003	0.082	0.99	0.265	0.26	0.006
Node 5	<0.01 (1)	0.01 (1)	0.28 (1)	0.10 (1)	0.02 (1)	0.03 (1)	0.45 (6)
(D<0.0001)	0.936	0.944	0.595	0.748	0.885	0.860	0.998

NOTE: Variant alleles at *Idh3,4* and *Mdh3,4* were combined into a single class.

TABLE 4. Nei's genetic distances among six samples of rainbow trout from the lower Laurentian Great Lakes. Key to sample abbreviations is given in Table 1.

	GH	NH	GR	SR	YC
BH	0.006	0.004	0.007	0.013	0.005
GH		0.001	0.002	0.006	0.002
NH			0.003	0.003	0.000
GR				0.007	0.004
SR					0.003

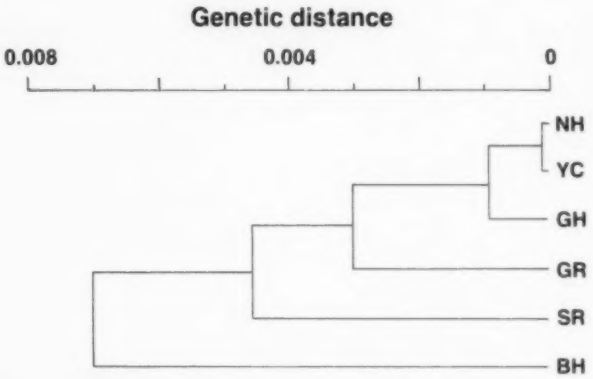

FIG. 2. Dendrogram (UPGMA) of genetic distances among six samples of rainbow trout from the lower Laurentian Great Lakes based upon 33 enzyme loci. Key to sample symbols is given in Table 1.

expressed (e.g. muscle) (Fig. 3C). This allele does not affect liver expression, as PGM1 enzyme is not usually present in that tissue (Fig. 3D). It is difficult to distinguish fish with *Pgm1-s(100/n)* genotypes from those with *Pgm1-s(100/100)* because both have PGM1 enzyme. Thus, only null allele homozygotes allow unambiguous detection of this variant. A single fish from the Ganaraska River progeny sample was a null allele homozygote (Table 2). This genotype was not detected in the Ganaraska hatchery sample. However, the null allele must have been present, but undetectable in the heterozygous state, because segregating families were produced from the hatchery stock the following year.

Phenotypic Correlates

A three-way ANOVA (PGM1 phenotype, female, male) with interaction (second and third order) indicated that Saugeen rainbow trout with liver PGM1 from 12 families were marginally longer than their full-sibs without activity. The third-order interaction term (PGM1*female*male) was significant and therefore was used as the error term in testing for PGM1, female, and male effects. Fish with liver PGM1 were marginally larger than fish without activity ($F_{[1,5]} = 3.42$, $P_{1\ tail} = 0.062$) (Table 5). No female and male effects were detected. The significance of the third-order interaction term was primarily due to PGM1*male effects ($F_{[5,415]} = 4.134$, $P = 0.001$) rather than PGM1*female effects ($F_{[1,415]} = 0.540$, $P = 0.463$).

The significant interaction between PGM1*males is apparently due to the effects of a single male. When the two families with this male (C7 and C13) were deleted from the analysis, the PGM1*male*female and PGM1*male interaction terms were no longer significant ($F_{[4,350]} = 1.857$, $P = 0.118$ and $F_{[4,350]} = 1.142$, $P = 0.337$, respectively). Importantly, the revised test of PGM1 against the within error term was highly significant ($F_{[1,350]} = 19.521$, $P < 0.001$), indicating significant effects of PGM1 on size.

Fish with muscle PGM1 (*100/100* or *100/n* genotypes) from four Ganaraska families differed in fork length but not in the number of asymmetric characters from those lacking activity (*n/n* genotypes). The variation for each variable was partitioned by two-way ANOVA into (1) family, (2) PGM1 phenotype (active or null), and (3) family × PGM1 phenotype interaction effects. Null allele homozygotes were significantly longer than fish with active alleles ($F_{[1,294]} = 6.678$, $P = 0.01$) (Table 6). Significant differences were detected between families ($F_{[3,294]} = 9.545$, $P < 0.001$) and interaction effects were marginal ($F_{[3,294]} = 2.501$, $P = 0.06$). Families and fish with different PGM1 phenotypes did not differ in asymmetry ($F_{[3,170]} = 1.646$, $P = 0.181$ and $F_{[3,170]} = 0.927$, $P = 0.337$, respectively).

These data show that variation in the amount of PGM is associated with phenotypic characteristics related to fitness. The phenotypic associations of PGM1 variation could be the direct effect of each locus (*Pgm1-s* or *Pgm1-t*) or a chromosomal segment that each locus is marking. The direct involvement of the enzyme locus predicts that the associations between genotypic

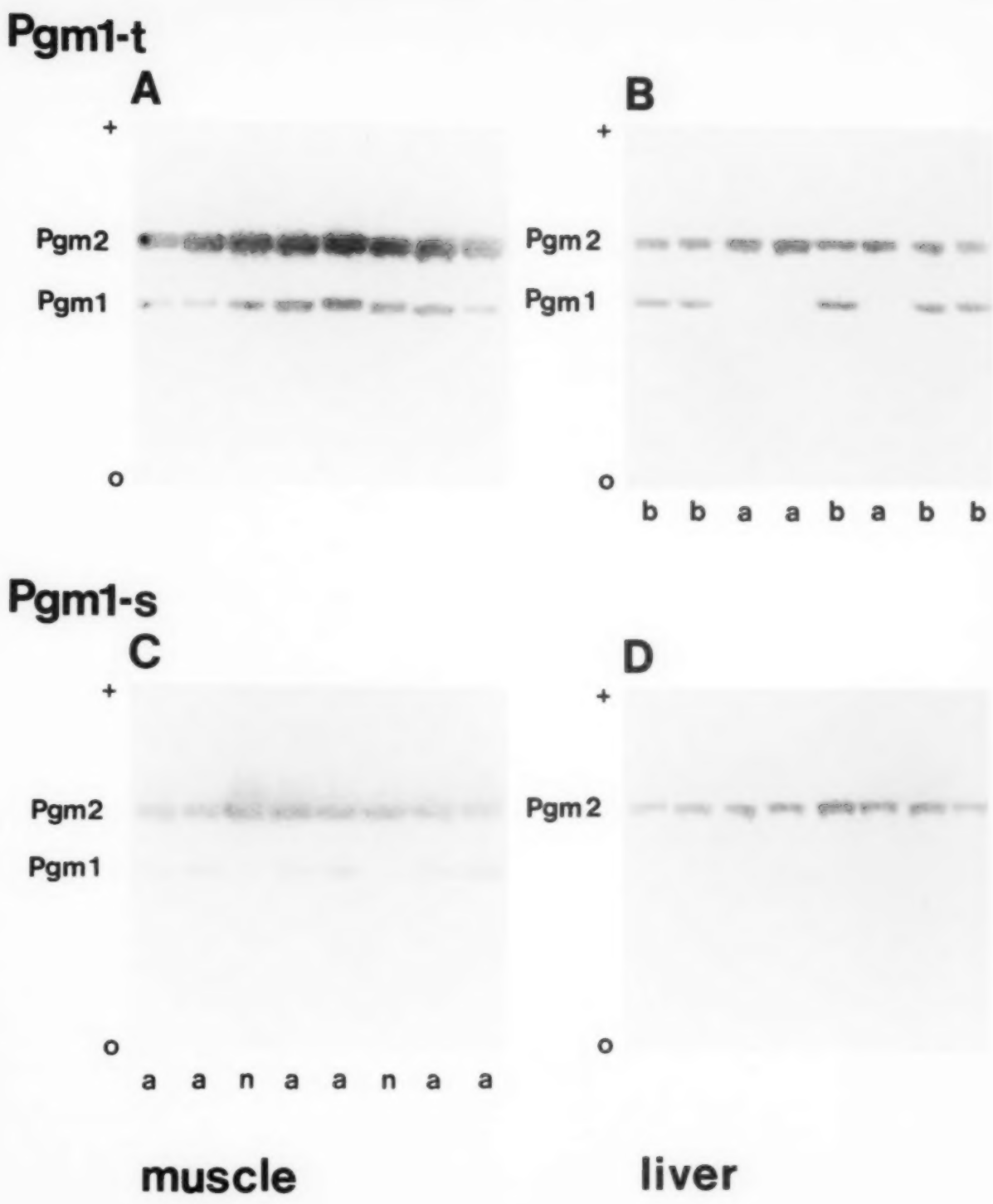

FIG. 3. Tissue-specific expression of *Pgm1-t* in (A) muscle and (B) liver and *Pgm1-s* in (C) muscle and (D) liver of rainbow trout. *Pgm1-t: b* = present, *a* = absent; *Pgm1-s: a* = active, *n* = null.

TABLE 5. Mean lengths (cm) of fish from 12 families of Saugeen rainbow trout segregating at *Pgm1-t*.

			Pgm1-t(a/a)		*Pgm1-t(a/b)*	
Family	Female	Male	Mean (SEM)	*N*	Mean (SEM)	*N*
C2	4	8	4.67 (0.09)	15	4.85 (0.07)	20
C3	4	20	4.62 (0.10)	24	4.79 (0.8)	24
C4	4	24	4.82 (0.10)	15	5.05 (0.10)	21
C5	4	34	4.80 (0.11)	16	4.88 (0.10)	20
C6	4	35	4.81 (0.13)	14	5.11 (0.06)	22
C7	4	36	4.79 (0.13)	14	4.77 (0.09)	21
C8	5	8	4.59 (0.11)	17	4.95 (0.08)	19
C9	5	20	4.96 (0.09)	18	5.02 (0.07)	18
C10	5	24	5.02 (0.13)	15	5.47 (0.06)	21
C11	5	34	4.85 (0.08)	16	4.99 (0.07)	20
C12	5	35	5.09 (0.06)	23	5.04 (0.12)	12
C13	5	36	5.01 (0.08)	19	4.54 (0.11)	15
Total			4.84 (0.03)	206	4.95 (0.03)	233

TABLE 6. Mean fork lengths (cm) and number of asymmetric meristic characters in fish from four families of Ganaraska rainbow trout segregating at *Pgm1-s*.

		Length		Asymmetry	
Family	Muscle PGM1	Mean (SEM)	*N*	Mean (SEM)	*N*
B36	Present	9.57 (0.23)	56	0.68 (0.17)	19
	Absent	10.61 (0.48)	19	0.68 (0.19)	19
B37	Present	9.76 (0.19)	61	0.68 (0.18)	18
	Absent	9.87 (0.29)	18	0.94 (0.17)	18
B40	Present	11.24 (0.19)	34	0.95 (0.18)	19
	Absent	11.09 (0.37)	19	1.21 (0.16)	19
B43	Present	10.33 (0.20)	62	0.94 (0.16)	33
	Absent	11.42 (0.25)	33	0.88 (0.14)	33
Total	Present	10.11 (0.11)	213	0.83 (0.09)	89
	Absent	10.86 (0.18)	89	0.92 (0.08)	89

variation and phenotype should be consistent regardless of genetic background (i.e. across families or strains) (Zouros and Foltz 1987). The marginally larger size of *Pgm1-t(b)* fish in the Saugeen strain is consistent with results observed in the Arlee strain of rainbow trout (Allendorf et al. 1983; Ferguson and Danzmann 1985; Ferguson et al. 1988a; Ferguson et al. 1990). The present analysis, however, detected significant interaction between size and genotype, suggesting that in some families, *Pgm1-t(b)* fish were smaller. The biological significance of the inversed relationship between PGM1 phenotype and length in the one family is difficult to evaluate because this result is possible by chance given the large number of families that have been analyzed in the various strains. However, physiological experiments with the Arlee strain are also consistent with the hypothesis that *Pgm1-t* variation influences the phenotype (Arnoson 1985; Ferguson et al. 1990).

The relationship between *Pgm1-s* variation and phenotype is not consistent with the direct involvement of the enzyme locus. According to the direct involvement hypothesis, *Pgm1-s(n/n)* fish should have been smaller, not bigger, because of reduced enzyme activity for metabolic processes. Thus, the larger size of *Pgm1-s(n/n)* fish suggests that the *Pgm1-s* locus is marking chromosomal segments that influence growth. However, regardless of whether the enzyme loci are directly involved, these data suggest that biochemical genetic variation is associated with traits likely to be important in the survival of stocked fishes.

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Summertime Bottom Temperatures on the Southeast Shoal of the Grand Bank, and Implications for Exchange Rates

John W. Loder

Physical and Chemical Sciences, Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada

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From spring to fall, the Southeast Shoal usually has the warmest bottom water on the entire Grand Bank of Newfoundland. Historical hydrographic data indicate that the elevated lower-layer temperatures are derived from the seasonal surface heat input, through vertical exchange across the developing thermocline in combination with the shallow depths of the Shoal. However, in some months, the surface fluxes required by the monthly changes in depth-integrated heat content over the Shoal exceed climatological flux estimates computed using bulk formulae, suggesting that the climatological estimates may be low. The seasonal evolution of the lower-layer temperature provides information on the rates of vertical exchange across the thermocline and horizontal exchange in the lower layer. A seasonal variation in the vertical exchange coefficient, with an approximately inverse relation to the vertical density difference, and significant horizontal exchange in summer are suggested. The associated rates are quantified with a crude axisymmetric model for the lower layer's monthly heat budget.

Du printemps à l'automne, c'est généralement sur le Platier que l'on observe l'eau de fond la plus chaude sur l'ensemble du Grand Banc de Tere-Neuve. Les données hydrographiques recueillies depuis longtemps indiquent que les températures élevées de la couche inférieure sont causées par l'apport saisonnier de chaleur superficielle, dû à l'échange vertical au travers de la thermocline qui se développe, et à la faible profondeur de ce banc. Toutefois, pendant certains mois, les flux superficiels nécessaires pour justifier les changements mensuels dans le bilan thermique intégré sur la profondeur au-dessus du banc dépassent les estimations des flux climatologiques calculés à l'aide de formules globales, ce qui indiquerait que ces estimations sont peut-être trop faibles. L'évolution saisonnière de la température dans la couche inférieure donne de l'information sur les taux d'échange vertical en travers de la thermocline et d'échange horizontal dans la couche inférieure. Il semblerait exister une variation saisonnière du coefficient d'échange vertical, avec une relation approximativement inverse à la différence dans la densité verticale, et un important échange horizontal en été. Les taux connexes sont quantifiés par un modèle axisymétrique grossier décrivant le bilan thermique mensuel de la couche inférieure.

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The Southeast Shoal is a broad (~100–200 km) and shallow (depths ~40–60 m) offshore area, situated near the Tail of the Grand Bank of Newfoundland and straddled by the outer boundary of Canada's 200-mile fishing zone (Fig. 1). During the spring–fall period of net surface heat input and increased buoyancy advection by the Labrador Current, a pronounced two-layer stratification develops over the Shoal, as over the remainder of the Bank (e.g. Petrie et al. 1991). A distinctive feature of the associated temperature field is the occurrence of (slightly) elevated lower-layer temperatures over the Shoal, such that it generally has the warmest summertime bottom temperatures of the entire Bank.

This bottom-temperature feature is of interest on several counts. Biologically, it may be a factor in (i) the location and timing of spawning by fish species such as capelin and yellowtail flounder which spawn on the southern Grand Bank (Fitzpatrick and Miller 1979) and may be influenced by water temperature (Carscadden 1978; Carscadden et al. 1989; Pitt 1970), (ii) the emergence and distribution of the larvae of these and other species which show spatial patterns similar to those in bottom temperature (Frank and Carscadden 1989; Frank et al. 1991), and (iii) the distributions of adult fish such as haddock (Templeman and Hodder 1965) which have a preferred temperature range. Physically, the variability in the intensity and location of the feature provides valuable information on vertical exchange rates across the pycnocline and on horizontal circulation and exchange rates in the Shoal's lower layer. In turn, these exchange rates may be important factors in the rich benthic and phytoplankton production over the Shoal (e.g. Anderson and Gardner 1986).

The purposes of this paper are (1) to provide a *climatological* (averaged over all years) description of the seasonal evolution of the relatively warm bottom water over the Southeast Shoal and (2) to point out the implications for exchange rates over the Shoal. Detailed descriptions of the current and hydrographic variability in particular years, primarily based on observations from a dedicated field study in 1986–89, will be presented elsewhere. Here, the temperature field over the southern Grand Bank is described using long-term monthly means, and possible origins for the elevated bottom temperatures are discussed. The main thrust of the paper then follows: a crude heat

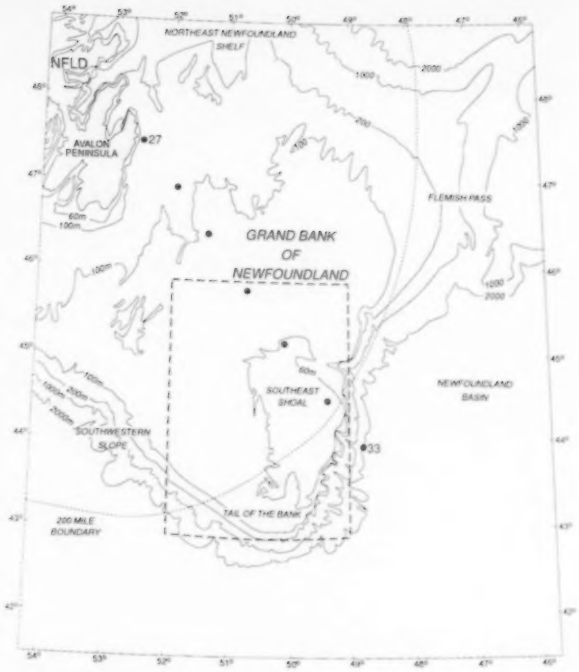

FIG. 1. Location map for the Southeast Shoal of the Grank Bank, with depths in metres. The broken box is the area in Fig. 3, and the solid circles are the station locations of the "St. John's – SE slope Grand Bank" hydrographic section (Fig. 6).

budget for the bottom water and its implications for exchange rates.

Climatological Temperature Distributions

The large-scale distribution of summertime bottom temperature over the Grank Bank is illustrated by the July–August temperatures at 50 m (Fig. 2), obtained from Drinkwater and Trites' (1986) monthly means. The cold intermediate layer (taken as $T < 0$°C) of the Northeast Newfoundland Shelf (Petrie et al. 1988) can be seen to extend around the Avalon Peninsula and towards Flemish Pass, following known branches of the Labrador Current (Petrie and Anderson 1983). Relatively "warm" water appears to be intruding onto the Grand Bank from the southwest, similar to that described by Templeman and Hodder (1965) for some spring seasons, although the spatial resolution of Drinkwater and Trites' areas is not adequate to make firm statements on its structure. It is clear, however, that cool temperatures dominate the Grand Bank's lower water column in summer.

To examine the climatological temperature field over the southern Grand Bank with increased spatial resolution, monthly temperature statistics (means, standard deviations, etc.) were obtained from the Marine Environmental Data Service (MEDS) of the Department of Fisheries and Oceans (Ottawa, Canada) for 0.5 × 0.5° squares in the area 43–46°N, 49–52°W. The statistics were computed at standard oceanographic depths (0, 10, 20, 30, 50, 75, 100 m, etc.) using the combined bottle and expendable bathythermograph data bases up to 1984. The monthly mean distributions at 50 m (Fig. 3) suggest that the

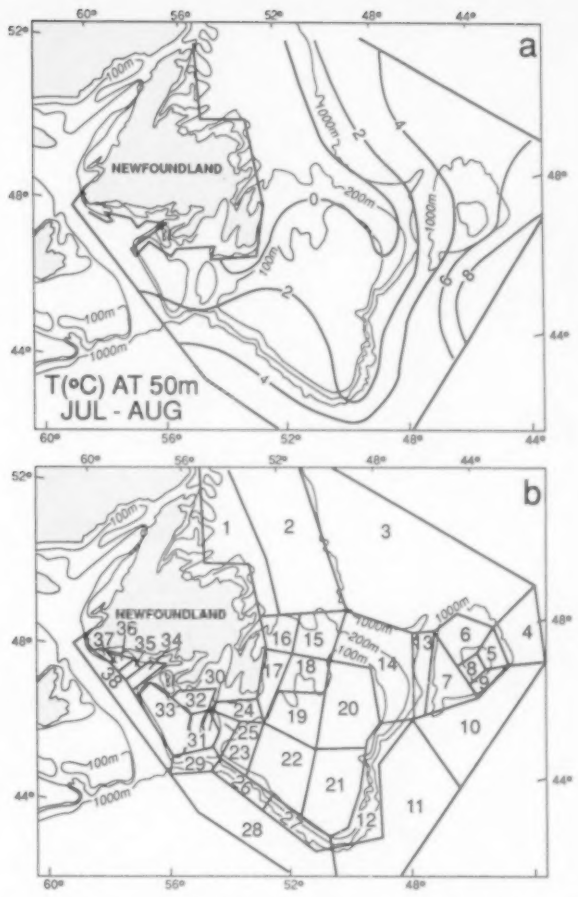

FIG. 2. (a) Average July–August temperature at 50 m on the Grand Banks of Newfoundland, based on Drinkwater and Trites' (1986) monthly means for the subareas in (b).

elevated bottom temperatures on the southern Grand Bank actually have two maxima: one over the southwestern slope in some months and another over the Southeast Shoal in most months. Indeed, the highest summertime temperatures at this depth occur over the Shoal (on average), with values peaking at over 4°C in July. The Shoal's temperature maximum is located slightly to the west of its shallowest part, however, perhaps related to the presence of cold intermediate-layer water along the eastern edge of the Bank. There is also a suggestion that the cool water extends farthest on-Bank near the Shoal's northeast corner, perhaps a signature of enhanced cross-shelf exchange over canyons (e.g. Kinsella et al. 1987).

The vertical distributions of temperature across the Shoal (Fig. 4) show thermal stratification developing in April and evolving into a pronounced two-layer structure by late summer with a thermocline between 10 and 30 m. The elevated bottom temperatures over the Shoal appear to include contributions from both the shoaling of the seafloor into the thermocline and a warming of the lower layer over the Shoal. There is little suggestion of a change in the upper-layer temperature associated with the warmer bottom water.

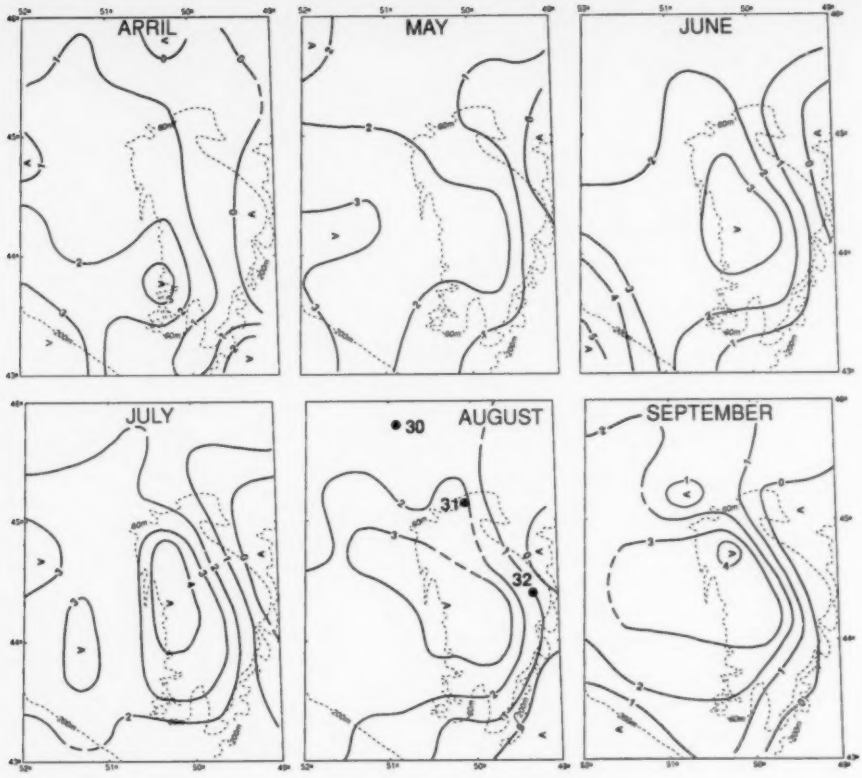

FIG. 3. Monthly temperature distributions at 50 m on the southern Grand Bank, based on MEDS monthly means for 0.5 × 0.5° squares. The area covered is indicated in Fig. 1. Broken portions of contours indicate interpolation through squares with no data. The location of the hydrographic section (Fig. 6) stations is included on the August map.

Possible Origins of Elevated Bottom Temperatures on the Southeast Shoal

The temperature distributions suggest two possibilities for the origin of the elevated summertime bottom temperatures over the Southeast Shoal: episodic intrusions of Slope Water onto the Bank (e.g. Templeman and Hodder 1965), followed by reestablishment of the cool Labrador Current flow around its perimeter, and vertical exchange of upper-layer heat across the thermocline (e.g. Petrie et al. 1991). A potentially distinguishing characteristic of these alternative hypotheses is the expected salinity of the bottom water, as suggested by temperature–salinity curves (Fig. 5) for the southeastern Bank and surrounding areas. Whereas the warm upper-layer water over the southeastern Bank (area 21) has a *lower* salinity than the underlying water, the warmer Slope Water offshore to the southwest (area 28) and southeast (area 11) has a *higher* salinity at 50 m than the lower-layer shelf water. Thus, a reduction in lower-layer salinity over the Shoal is expected to be associated with vertical mixing and an increase associated with Slope Water intrusions.

To examine whether the Shoal's bottom-temperature maximum has a distinct salinity signature, monthly statistics of salinity were also obtained from MEDS for 0.5 × 0.5° squares. The resulting horizontal salinity distributions (not shown) are much more variable (and probably considerably less reliable) than the temperature distributions, presumably due to the substantially smaller salinity data base and possible aliasing of the higher interannual (compared with seasonal) variability in salinity (Loder and Ross 1988). There is, however, no suggestion of elevated salinities associated with the Shoal's elevated bottom temperatures. There is some suggestion that the bottom salinity over the Shoal in July is lower than in surrounding areas, supportive of a vertical mixing origin, but the salinity distribution is not adequately resolved to reach firm conclusions.

Another historical data set which suggests that the bottom-temperature maximum is not associated with higher salinity water is the annual August hydrographic section "St. John's – SE slope Grand Bank" (see Fig. 1 for station locations) occupied by the St. John's Biological Station in most years between 1949 and 1979 (Templeman 1975). Warmer bottom temperatures over the Shoal are apparent in most individual sections (not shown) and in the average temperature section for 1951–65 and 1969–71 (Fig. 6), without elevated bottom salinities (reduced bottom salinities are apparent in some years). There is no significant correlation between the interannual anomalies of bottom temperature and salinity at station 31 and a negative correlation ($r = -0.71$, $n = 20$) significant at the 99% level (Student's t-test) at station 32. Although the latter is consistent with the vertical mixing hypothesis, these stations are poorly

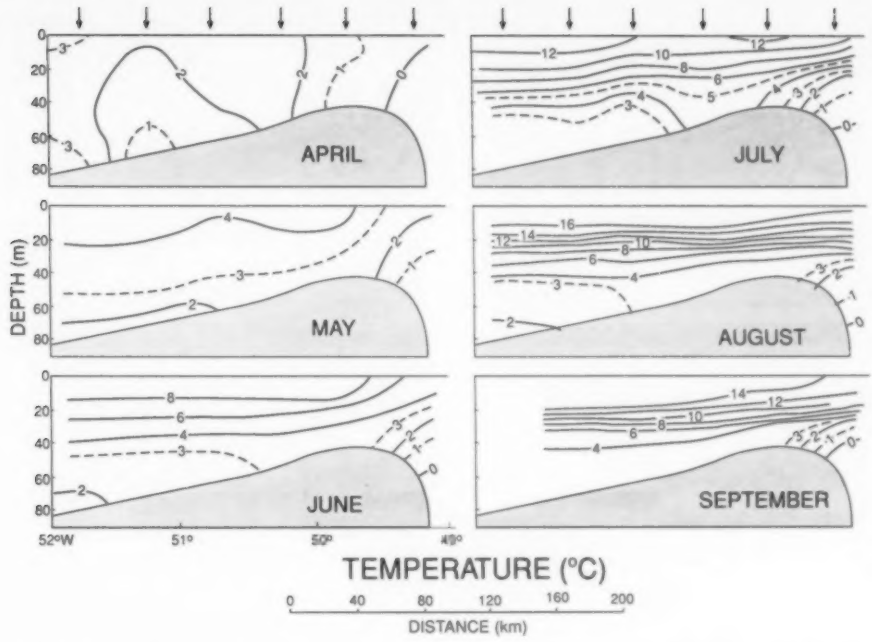

FIG. 4. Monthly sections of temperature across the Southeast Shoal, based on MEDS monthly means for 0.5 × 0.5° squares between 44 and 44°30′N. The arrows indicate the midposition of each square.

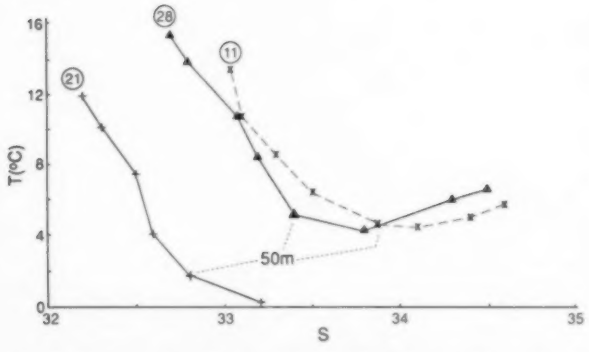

FIG. 5. Temperature–salinity curves for the July monthly means in Drinkwater and Trites' (1986) subareas (Fig. 2b): Southeastern Grand Bank (21), Southwestern Newfoundland Basin (11), and Southern Offshore (28). Values are plotted at standard depths, with the 50-m values indicated.

located for sampling the temperature maximum (see August map in Fig. 3) and firm conclusions cannot be reached.

The historical data thus suggest that, although Slope Water intrusions may be a major factor in the elevated 50-m temperatures on the southwestern slope of the Grand Bank (e.g. April–June distributions in Fig. 3), they are not the primary contributor to the bottom-temperature maximum over the Southeast Shoal. This conclusion is also supported by observations (under analysis) taken during the 1986–89 field study.

Monthly Heat Budgets

In this section, aspects of the monthly heat balance of the waters over the Southeast Shoal are examined. Fixed areas (Fig. 7) were chosen on the basis of the 50-m July temperature distribution (Fig. 3): a "central core" approximating the area of the warm bottom water and an "outer band" approximating the cooler ambient water. Monthly statistics (over all years) of the temperature (and salinity) at standard depths were obtained from MEDS for these areas and used in the analyses. Time–depth (t–z) plots of the monthly mean temperatures (Fig. 8) show the spring–summer evolution of a two-layer stratification in both areas, followed by fall destratification.

Comparison with Surface Fluxes

Before examining the lower layer's budget, the monthly changes in depth–integrated heat content over the Shoal are compared with climatological information (Bunker 1976; Isemer and Hasse 1987) on the net heat flux across the sea surface (Fig. 9). Readily available monthly surface fluxes are used, computed from bulk formulae by Bunker (1976) for the zonal band 45–46°N, 50–60°W overlying the continental shelf (including the Grand Bank) south of Newfoundland and by Isemer and Hasse (1987) for the area 48–50°N, 51–53°W just north of the Grand Bank. Both of these areas are slightly north of the Southeast Shoal, but Bunker's and Isemer and Hasse's maps indicate that, if anything, the spring–fall fluxes into the ocean over the southern Grand Bank are *less* than in the areas to the north.The fluxes in the Isemer and Hasse atlas are based on Bunker's data but computed with revised formulae for some components, since other studies (e.g. Smith and Dobson 1984) have indicated that the Bunker fluxes underestimate the heat input to the ocean (by an annual average of 70 $W \cdot m^{-2}$ in the Labrador Sea).

The climatological fluxes are in rough agreement with the surface fluxes required by the month-to-month changes in heat

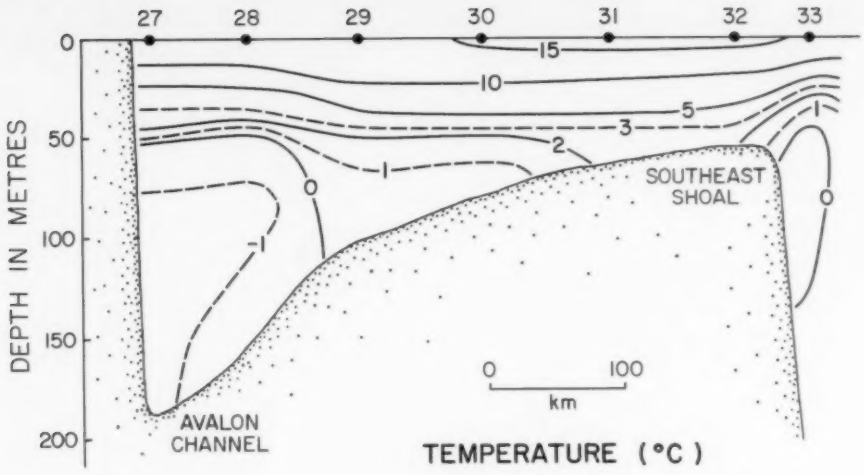

FIG. 6. Average temperature on the August section "St. John's – SE Slope Grand Bank" for the years 1951–65 and 1969–71 (from Templeman 1975). See Fig. 1 and 3 for station locations.

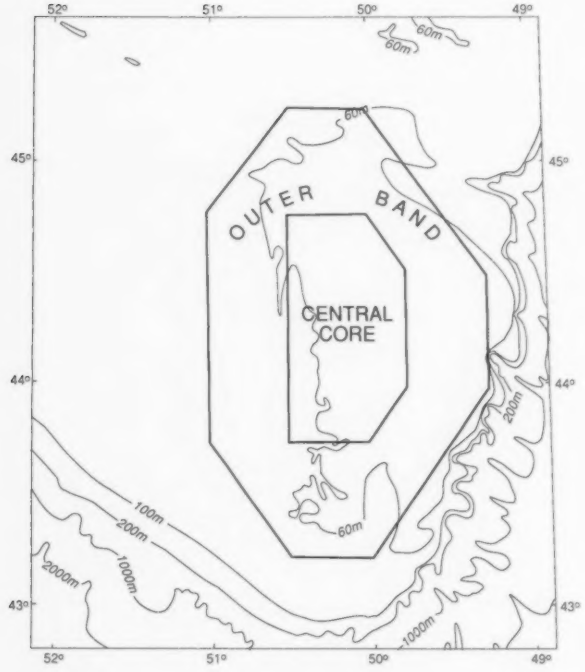

FIG. 7. "Central core" and "outer band" areas used in the heat budgets.

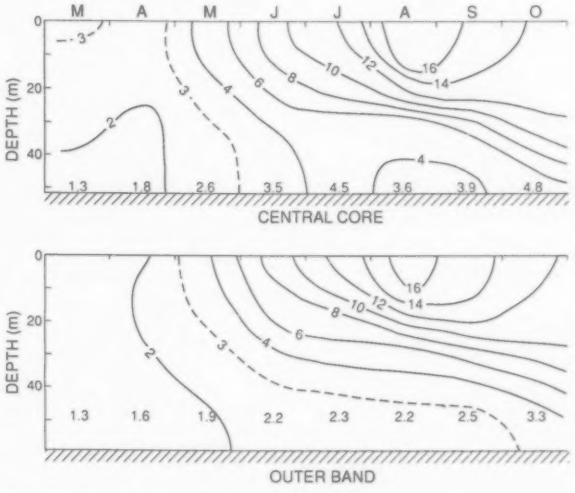

FIG. 8. Time–depth evolution of monthly mean temperature in the central core and outer band (Fig. 7). The monthly means at 50 m are shown. The standard deviations and errors of the 50-m means are typically 1.1 and 0.2, respectively.

content in the central core and outer band (Fig. 9), neglecting contributions from horizontal exchange. There are also some notable discrepancies. The average temperature in the central core is generally higher than in the outer band (Fig. 8), suggesting that horizontal exchange would probably contribute to heat export from the Shoal and thus require that the actual surface fluxes exceed the estimates from the heat content changes. In contrast, the intercomparison (Fig. 9) indicates that, for May–June and July–August, the climatological fluxes are less than those required by the heat content changes. Considering that Isemer and Hasse's (1987) fluxes for the southern Grand Bank are at the lower end of the range shown in Fig. 9, there is a suggestion that, in some months, either their fluxes for the Bank are low (perhaps due to horizontal smoothing with the estimates for the ambient and warmer deep-ocean waters) or a significant horizontal import of heat to the Shoal occurs (assuming that the heat content estimates are reliable). The suggestion of heat import comes back to a possible contribution from Slope Water intrusions from the southwest (e.g. Fig. 3) or from an eastward drift of relatively warm surface water (e.g. Fig. 4); however, numerical model predictions (Greenberg and Petrie 1988; Hukuda et al. 1989) and moored measurements (Ross et al. 1988) of a weak westward drift (0.01–0.03 $m \cdot s^{-1}$) over the Bank do not support such a drift.

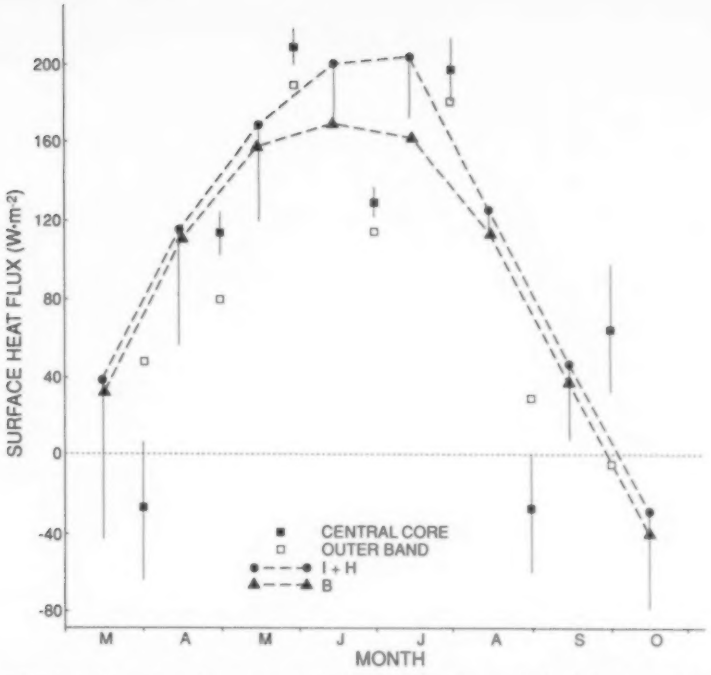

FIG. 9. Surface heat fluxes (into the ocean) required by monthly changes in depth-integrated heat content in the central core and outer band, together with climatological fluxes from Isemer and Hasse (1987; I + H) and Bunker (1976; B). The depth-integrated heat content is computed as a weighted sum of the monthly means for standard depths. The standard errors of the fluxes implied by the central-core heat content changes, computed assuming that variations about the individual-level monthly means are uncorrelated (so that each error is taken as the square root of the sum of the squares of the weighted individual-level standard errors), are shown. The I + H fluxes for both 48–50°N, 51–53°W (solid circles) and 45°N, 50°W (lower end of vertical line; estimated from their Charts 117–128) are included.

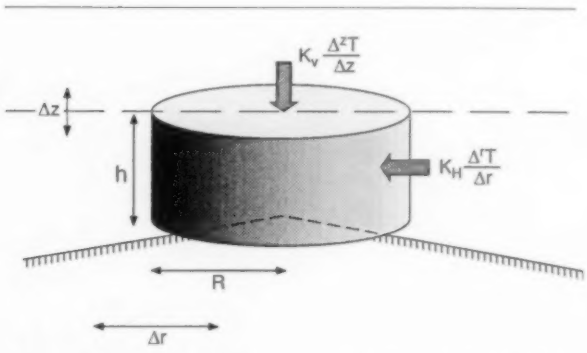

FIG. 10. Schematic of pillbox representing the central core's lower layer.

Lower-Layer Budget

The seasonal evolutions of temperature in the central core and outer band (Fig. 8) show considerable similarity, but there are two significant differences in the lower layer (e.g. the 50-m temperatures). First, the spring–summer warming rate in the central core is higher than in the outer band, resulting in the central-core bottom temperatures being about 2° warmer from July to October (as in Fig. 3 and 4). Second, while the outer-band bottom temperatures show an approximate monotonic increase between spring and fall, the March–July warming of the central-core bottom water is interrupted by cooling between July and August (by an amount exceeding twice the sum of the July and August standard errors). The outer-band variation is qualitatively consistent with vertical exchange being the only contributor to the heat budget, but the central-core variation indicates significant horizontal exchange in the lower layer, in July–August at least. Note that this suggests a horizontal export of heat, which would require that the actual surface heat flux be greater than that required by the observed head content change (neglecting horizontal fluxes in the upper layer). Since the climatological surface fluxes for July–August (Fig. 9) are lower rather than higher than the heat content requirements, this supports the questions raised earlier regarding the climatological fluxes.

To make quantitative statements about the vertical and horizontal exchange rates implied by these areal-averaged temperature variations, the central core's lower layer (taken as the water column below 25 m) is approximated by a pillbox (Fig. 10) of equivalent area, average thickness, and volume. Assuming that the vertical temperature flux into the pillbox (i.e. across the thermocline) can be parameterized by a vertical exchange coefficient K_V times the temperature gradient between 20 and 30 m, and the horizontal flux across the pillbox's sides can be parameterized by a horizontal exchange coefficient K_H times the lower-layer temperature gradient between the central core and outer band, the month-to-month

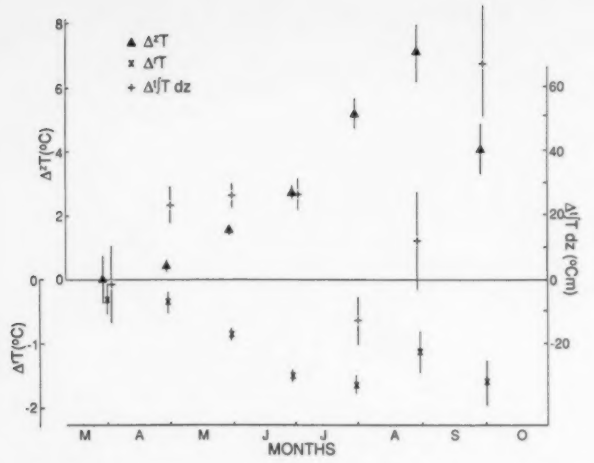

FIG. 11. Month-to-month changes ($\Delta' \int T\, dz$) in vertically integrated temperature in the central core's lower layer, and averages (across pairs of months) of vertical ($\Delta^z T$) and horizontal ($\Delta' T$) temperature differences. The lower-layer temperatures are computed as a weighted sum of the 30- and 50-m monthly means. The standard errors, computed on the assumption that variations about the monthly means for individual depths or areas are uncorrelated, are also shown.

changes in vertically integrated temperature in the pillbox satisfy

$$(1) \qquad \frac{\Delta' \int T\, dz}{\Delta t} \pi R^2 = K_V \frac{\Delta^z T}{\Delta z} \pi R^2 + K_H \frac{\Delta' T}{\Delta r} 2\pi R h.$$

Here, R and h are the pillbox radius (44 km) and thickness (26 m), $\Delta' \int T\, dz$ is the change in the central core's integrated temperature during a month Δt, $\Delta^z T$ is the difference in central-core temperature over the vertical interval Δz between 20 and 30 m, and $\Delta' T$ is the difference between the (volume-averaged) lower-layer temperatures in the outer band and central core, over the distance Δr (38 km) between the band's and core's midcircles (based on area). Using the MEDS monthly means to evaluate $\Delta^z T$, $\Delta' T$, and $\Delta' \int T\, dz$, (1) yields a simple relation between K_V and K_H for each month-to-month interval:

$$(2) \qquad K_V = \frac{\Delta z}{\Delta t} \frac{\Delta' \int T\, dz}{\Delta^z T} - \frac{2h}{R} \frac{\Delta z}{\Delta r} \frac{\Delta' T}{\Delta^z T} K_H.$$

Although some of the assumptions (e.g. axisymmetric horizontal exchange) leading to (2) may not be strictly valid on the Southeast Shoal (see later), this relation provides a framework for obtaining first estimates of exchange rates. Note that spatial variability in the processes (e.g. Labrador Current along eastern bank edge) contributing to the outer-band temperature field do not invalidate the assumptions leading to (2), although spatial variations in the exchange rates *between* the central core and outer band are not resolved by K_H. Furthermore, the exchange represented through K_H can include advective as well as diffusive contributions, although K_H would not have a meaningful physical interpretation in the absence of some degree of axisymmetry in the temperature field.

As seen in the earlier figures, the month-to-month values of $\Delta^z T$, $\Delta' T$, and $\Delta' \int T\, dz$ for March–October (Fig. 11) indicate

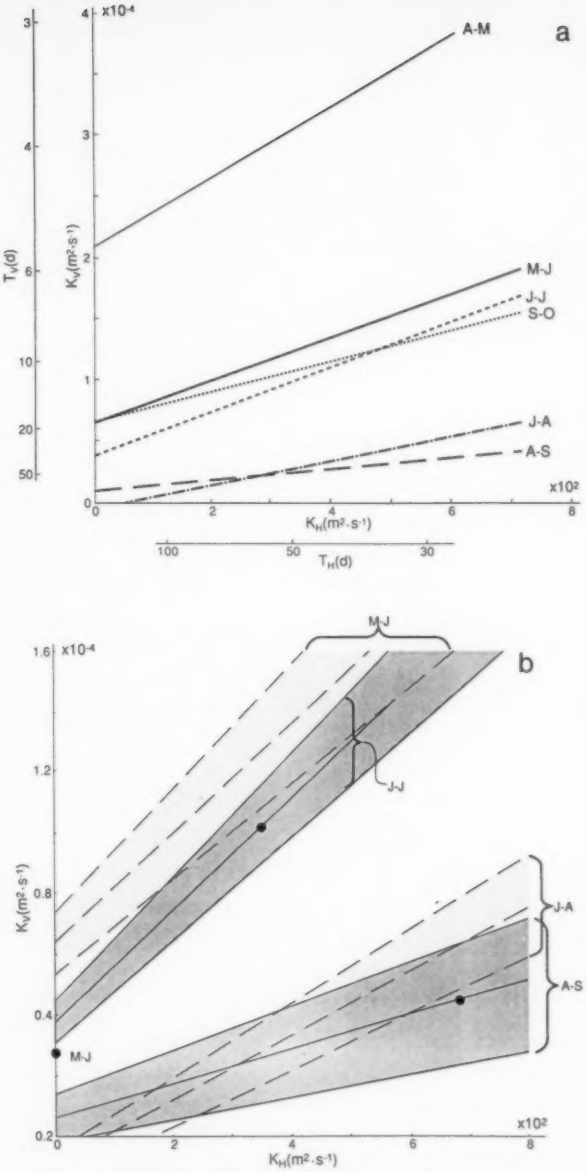

FIG. 12. (a) Monthly relations between K_V and K_H from the lower-layer budgets for April–October. The corresponding time scales for exchange across Δz and Δr are also shown. (b) Range of uncertainty in the K_V–K_H solutions for May–September associated with variability in the temperature data, computed with the standard errors (Fig. 11) added or subtracted from the monthly means. The solid circles represent the upper-bound K_V/K_H values from (4).

a pronounced seasonal development of the thermocline, a smaller seasonal increase in the horizontal temperature difference, and increases in the central core's lower-layer temperature in most months (with July–August being an exception). The standard errors indicate that most of the computed means for April–October have some significance.

The K_V–K_H relations inferred from the temperature data (Fig. 12) lie in limited (but variable) portions of K_V–K_H space.

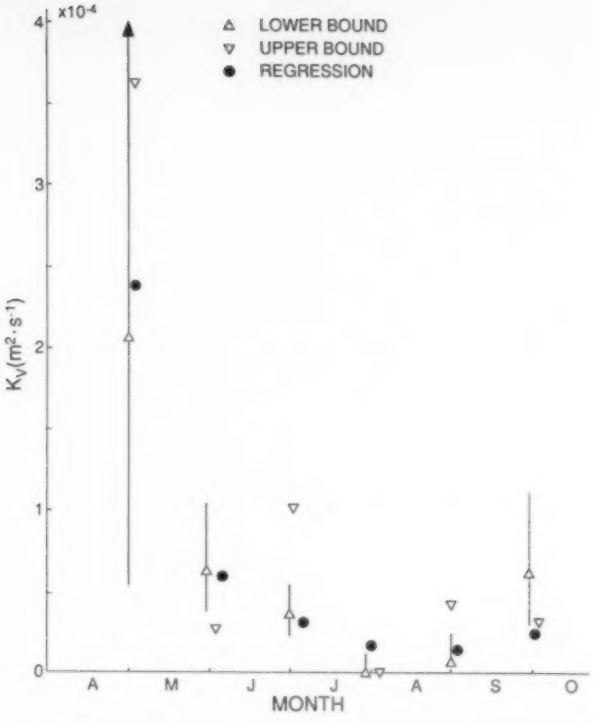

FIG. 13. Monthly estimates of K_V from the lower-layer budget (lower bound), from the upper-layer budget (upper bound), and from the best-fit (regression) to the lower-layer budget assuming constant K_H and K_V dependent on $\Delta\rho$ ($n = 1$).

The lower-layer heat budget can be satisfied by values of K_V as low as order 10^{-5} m^2·s^{-1} during July–September and order 10^{-4} m^2·s^{-1} during May–July and September–October, but the appropriate values depend on the horizontal exchange rates. The possible solutions range from the one-dimensional "slow-exchange" limit of insignificant horizontal exchange to the two-dimensional "fast-exchange" limit of the vertical heat flux being largely exported from the lower layer by horizontal exchange. Further information or assumptions are required, however, to identify the values appropriate to the Southeast Shoal.

These solutions may also be interpreted in terms of the time scales T_V and T_H required for exchange across the distances Δz and Δr. For given exchange rates, the corresponding time scales depend on the specific exchange problem (e.g. Loder et al. 1988), but they are of order $T_V \cong (\Delta z)^2/K_V$ and $T_H \cong (\Delta r)^2/K_H$. For the present values of Δz and Δr, these scales are also shown in Fig. 12a.

Bounds on K_V and K_H

The lower-layer budgets provide *lower* bounds for K_V and K_H. For cases when the solution (Fig. 12) intercepts the K_V axis (as is typical except for July–August), the intercept provides a nonzero lower bound for K_V and horizontal exchange is not required (lower bound of $K_H = 0$). The resulting lower-bound K_V's show a distinct seasonal variation, reaching a minimum in summer (Fig. 13). On the other hand, in the case of July–August when the solution intercepts the K_H axis, horizontal exchange is required with a lower-bound K_H value near 90 m^2·s^{-1} and vertical exchange is unnecessary (lower bound of $K_V = 0$).

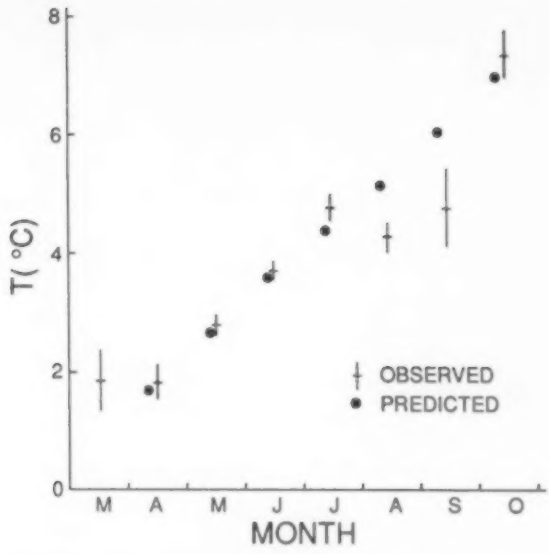

FIG. 14. Lower-layer temperatures predicted by (1) using the constant K_H and seasonally varying K_V values ($n = 1$) inferred from the regression analysis. Observed values with standard errors are also shown. The prediction used the observed March value as the initial condition.

Similarly, monthly budgets for the central core's upper layer can be used in conjunction with the climatological surface heat fluxes to obtain crude *upper* bounds on the vertical exchange rates. Neglecting horizontal exchange, the month-to-month changes in vertically integrated temperature in the upper layer satisfy

$$(3) \qquad \frac{\Delta' \int T\,dz}{\Delta t}\pi R^2 = \frac{Q}{\rho\, c_p}\pi R^2 - K_V \frac{\Delta^z T}{\Delta z}\pi R^2$$

where Q is the surface heat flux, ρ is the density, c_p is the specific heat, $\Delta' \int T\,dz$ now refers to the upper layer, and K_V and $\Delta^z T$ remain appropriate to the 20- to 30-m interval. Rearranging, we obtain

$$(4) \qquad K_V = \frac{\Delta z}{\rho\, c_p}\frac{Q}{\Delta^z T} - \frac{\Delta z}{\Delta t}\frac{\Delta' \int T\,dz}{\Delta^z T}$$

which allows estimation of K_V from the excess heat after the upper-layer budget is satisfied. Since the monthly mean upper-layer temperatures are higher in the central core than in the outer band, the first expectation would be for the inclusion of horizontal exchange to result in a horizontal export of heat in the upper layer, resulting in the K_V's estimated from (4) being upper bounds.

The upper-bound K_V's obtained from (4) using Isemer and Hasse's (1987) surface fluxes for 48–50°N, 51–53°W (Fig. 9) are compared with the lower-bound values in Fig. 13. There are similar seasonal variations with discrepancies of comparable magnitude to the uncertainties, suggesting that the actual values are close to the lower bounds from the lower-layer budget. However, some discrepancies between the surface fluxes and heat content changes are again apparent, with the

upper-layer budget (3) suggesting that no heat is available to the lower layer in July–August and not enough to account for minimum requirements ($K_H = 0$) in May–June and September–October.

The upper bounds on K_V (Fig. 13) also imply upper bounds on K_H. These are indicated in Fig. 12b for May–September. Collectively, the budgets imply little lower-layer horizontal exchange in May–June, little vertical exchange in July–August, and K_V and K_H values of up to 1×10^{-4} m^2·s^{-1} and several hundred metres squared per second, respectively, for June–July and August–September.

Seasonal Variation in K_V

Comparison of Fig. 13 and 11 indicates that the seasonal variation in K_V is approximately inversely related to the seasonal variation in $\Delta^z T$, an index of the thermocline strength. This relation is qualitatively similar to that in common parameterizations of the vertical diffusion coefficient (e.g. Blumberg 1977; Henderson-Sellers 1982), which have an inverse dependence on the amount of stratification (usually through a Richardson Number). To quantify this in the present situation, K_V is taken to have the form $K_V = K_o\,(\Delta\rho_o/\Delta\rho)^n$ where $\Delta\rho$ is the density difference between 20 and 30 m, K_o and $\Delta\rho_o$ are the values of K_V and $\Delta\rho$ in a particular month-to-month interval (taken as July–August), and n is an unknown parameter. This reduces the determination of K_V's seasonal variation to two constants (K_o and n). Substituting in (1), rearranging, and taking K_H to be constant as a first approximation, a set of simultaneous equations in K_o, n, and K_H is obtained:

$$(5) \qquad A_i = K_o B_i + K_H C_i \qquad (i = 1, m)$$

where $A_i = \Delta^t \int T\,dz$, $B_i = \Delta^z T\,(\Delta t/\Delta z)\,(\Delta\rho_o/\Delta\rho)^n$, $C_i = \Delta^t T\,(2h\Delta t)/(R\Delta r)$, subscript i denotes the particular month-to-month interval, and m is the number of intervals/equations (seven for March–October). With these assumptions, the previously underdetermined set of m equations in $2m$ unknowns (K_V, K_H for each interval) is reduced to the overdetermined set of m equations in three unknowns.

To determine the values of K_o, n, and K_H which give the best fit to the lower-layer heat content changes and temperature differences (Fig. 11), multiple linear regressions of A_i against B_i and C_i were performed, with B_i evaluated for various plausible choices of n. Monthly mean MEDS data were used to evaluate $\Delta\rho$ and the various intervals were weighted according to the number of lower-layer temperature observations in the central core (using the weighted regression option of the BMDP (1983) statistical software package). Additional "intervals" (simultaneous equations) were obtained from the lower-layer salinity budgets corresponding to (1), allowing various choices of m.

For the cases (i.e. choices of m and n) yielding results consistent with the assumptions (i.e. positive values of K_o and K_H), the highest multiple correlation coefficient was generally obtained with n near 1. However, the results are somewhat inconclusive, with the best-fit values of n, K_o, and K_H dependent on m and good fits also being obtained with corresponding negative exchange coefficients. Presumably this indeterminancy indicates some degree of data inadequacy and/or inappropriate assumptions. The inclusion of the salinity budgets did not improve the determination, consistent with the limited quantity and perhaps quality of the salinity data.

As an example of the inferred coefficients, the values of K_V obtained for $n = 1$ in the case of temperature budgets for March–October ($m = 7$) are shown (Fig. 13). In this case, the values of $n = 1$ and 1.25 gave the highest correlation coefficient (0.874), with regression coefficients (and associated standard errors) of $K_o = (0.18 \pm 0.10) \times 10^{-4}$ m^2·s^{-1} and $K_H = 20 \pm 130$ m^2·s^{-1} for $n = 1$. These values are typical of those obtained for other cases, with the July–August vertical coefficient being near 0.2×10^{-4} m^2·s^{-1} and the "best-fit" constant horizontal coefficient being less than 100 m^2·s^{-1} and not significantly different from zero. The regression results thus support a seasonally varying K_V (e.g. Fig. 13) with an approximately inverse dependence on the density difference across the thermocline.

The predicted lower-layer temperatures (Fig. 14) for the "best-fit" values of the exchange coefficients show a smooth seasonal increase with a net spring–fall change in approximate agreement with that observed. However, as expected for an overall best fit and the specified K_V dependence, the observed interruption in warming in July–August is not predicted. Using the best-fit vertical exchange coefficient in (1), the K_H values required by the observed temperatures are 250 and 130 m^2·s^{-1}, respectively, for July–August and August–September.

With the availability of detailed information on current variance from the 1986–89 field study, seasonal variations in both K_V and K_H associated with variable currents could be explored following the above approach. However, the usefulness of this effort is questionable without an improved representation of horizontal exchange, including relaxation of the assumption of axisymmetry and explicit inclusion of mean horizontal advection.

Discussion and Conclusions

The historical temperature data from the southern Grand Bank show the development of an area of relatively warm bottom water over the Southeast Shoal in spring and its persistence through summer and into fall. In addition to the feature's potential significance to spawning and larval and adult fish distributions, it provides valuable information on exchange rates over the Shoal.

The most likely origin for the elevated lower-layer temperatures is downward vertical mixing of the seasonal surface heat input. Some of the temperature increase can be explained from the reduced thickness of the lower layer over the Shoal and spatially uniform vertical mixing, but the possibility of enhanced vertical mixing over the Shoal is raised. There is rough agreement between the surface heat fluxes implied by the monthly changes in depth-integrated heat content and climatological fluxes computed from bulk formulae, but there is also a suggestion that the climatological fluxes are low in some months. The most likely effect of horizontal exchange (heat export) would amplify the discrepancies even further.

The available salinity data do not support a major contribution to the Shoal's warm bottom water from the alternative hypothesis of Slope Water intrusions, although there is evidence for such intrusions on the southwestern slope of the Grand Bank (Templeman and Hodder 1965). However, the salinity data are limited in quantity and possibly quality. The Southeast Shoal feature is a good example of a shelf bottom-water feature whose origin and interannual variability would be elucidated with more high-quality salinity data.

In contrast with the case of vertical diffusion only (e.g. Petrie et al. 1991), where the phase lag of the seasonal temperature cycle increases with distance below the surface and the bottom temperature is maximum in fall–winter, the bottom temperature on the Southeast Shoal reaches a weak secondary maximum in July, *preceding* the August primary maximum in surface temperature. This implies that there is significant horizontal exchange between the lower layer over the Shoal and the sur-

rounding water during summer, in combination with a reduction in vertical exchange. The crude monthly heat budgets for the Shoal's lower layer quantify the associated exchange rates in terms of average horizontal (K_H) and vertical (K_V) exchange coefficients. The budgets imply a summertime decrease in K_V with an approximately inverse relation to the vertical density gradient, K_V values in the range $(0.1–1.0) \times 10^{-4}$ $m^2 \cdot s^{-1}$ between May and September, and K_H values less than 100 $m^2 \cdot s^{-1}$ on average but perhaps higher in July–September.

Caution must be used, however, in interpreting these coefficients beyond the context of the present monthly averaged budgets: K_V is a coefficient which, multiplied by the average temperature difference between 20 and 30 m, yields the average vertical temperature flux; K_H is a coefficient which, multiplied by the average lower-layer temperature difference between the Shoal and surrounding area (distance of 40 km), yields the average radial temperature flux. Furthermore, the representation of the Shoal by axisymmetric fixed areas and the use of a spatially uniform K_H are crude approximations which result in a highly averaged description in space, as well as time. In particular, the omission from the model of a specific advection term and the assumption of axisymmetric horizontal exchange mean that the contributions from diffusion and mean and variable advection are not discriminated, and the observed differences in temperature (and probably exchange) between the Shoal's western and eastern sides are not resolved. Nevertheless, the model provides a quantitative description of the combined effects of all contributions to exchange, and it is encouraging that the K_V values are of comparable magnitude (although somewhat smaller) than the $(0.7–3) \times 10^{-4}$ $m^2 \cdot s^{-1}$ values computed by Loder et al. (1988) for the Southeast Shoal in June–August, using semiempirical parameterizations and moored current and hydrographic measurements.

The budget results allow further characterization of the Southeast Shoal in terms of integral time scales for vertical and horizontal exchange (Loder et al. 1988). As indicated in Fig. 12a, the early spring results suggest a vertical exchange time of order several days, while a horizontal exchange time of (at least) several tens of days is likely. Thus, vertical redistribution should be relatively rapid compared with horizontal replacement of Shoal waters in early spring. In summer, however, the results suggest that the vertical exchange time becomes of order 50–100 d, probably comparable with the horizontal exchange time. This is in contrast with banks such as Browns and Georges in the tidally energetic Gulf of Maine where relatively rapid vertical exchange occurs year-round (Loder et al. 1988).

Biological implications of the Southeast Shoal exchange rates might include slower (compared with Browns and Georges banks) exchange of nutrients, eggs, and larvae across the thermocline in summer, more classical seasonal variations in primary production with pronounced spring and fall blooms, an extended residence time for immotile organisms, particularly in May–July, and a supply of inorganic lower-layer nutrients to the central Shoal before transfer to the upper layer. Some suggestion of the latter is apparent in Anderson and Gardner's (1986) observations which show nutrients from the (eastern) Bank edge extending onto the Shoal below the thermocline and associated enhanced chlorophyll concentrations in the lower layer.

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PERSPECTIVES

Locally Based Water Quality Planning: Contributions to Fish Habitat Protection

Evelyn Pinkerton

School of Community and Regional Planning, University of British Columbia, 6333 Memorial Road, Vancouver, B.C. V6T 1W5, Canada

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Successful fish habitat protection occurs in areas of urban/industrial development when responsible citizens in rural watersheds can produce and implement local water quality plans binding on all agencies. In 1985, legislation in the state of Washington, USA, authorized a central planning agency — the Puget Sound Water Quality Authority — to initiate local watershed planning exercises through counties or other local agencies. The essential elements of community mobilization to the goals and activities of water quality planning were analyzed by comparing key factors in highly successful planning processes with factors in less successful ones. This permitted generalizations about basic organizing principles, educational procedures, and techniques of consensus building in the planning and implementation of water quality rehabilitation and protection for watersheds. The analysis contributed to a general theory of how and why community participation can improve the effectiveness of fish habitat protection.

Dans les régions de développement urbain et industriel, l'habitat des poissons est bien protégé lorsque des habitants responsables dans les zones rurales des bassins hydrographiques peuvent formuler et mettre en oeuvre des plans de protection de la qualité de l'eau mettant à contribution tous les organismes concernés. En 1985 dans l'État de Washington (É.-U.), un organisme de planification central, la *Puget Sound Water Quality Authority*, était autorisé par la loi à entreprendre la planification à l'échelle des bassins hydrographiques par l'entremise des administrations de comté ou d'autres organismes locaux. Afin d'analyser les grands éléments de la mobilisation de la collectivité en pour la réalisation des objectifs et des activités de planification, nous avons comparé les principaux facteurs des processus de planification qui ont réussi à ceux qui ont donné de moins bons résultats. Il a ainsi été possible d'énoncer les règles générales de l'organisation, des processus d'éducation et des moyens de faire le consensus dans la planification et la mise en oeuvre de la restauration et de la protection des bassins hydrographiques. Cette analyse s'inscrit dans une théorie générale portant sur les raisons pour lesquelles la participation de la collectivité peut augmenter l'efficacité de la protection de l'habitat des poissons et sur la façon dont cette amélioration se fait.

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Although habitat protection is a key component of fisheries management, it has seldom received a large share of management attention. Harvest management, allocation, enforcement of harvest regulations, and even hatchery production receive more funding and staff time than habitat protection.[1] Especially in regions undergoing industrial or urban expansion, habitat protection agencies tend to have inadequate staff and data, while industrial activities harmful to fish habitat tend to have more support from other government agencies and seemingly the public at large.

Yet without adequate habitat protection, there will be no sustainable harvest of fisheries resources (Williams et al. 1989). The habitat protection innovations described in this paper are of special interest because they occur in areas of rapid urban and industrial expansion. They can thus be considered successes in the worst-case scenario. This analysis focuses primarily on *why* and *how* the strategies adopted are workable and effective.

I analyze locally based watershed planning exercises which involve communities in the planning and implementation of water quality protection. Watershed planning focuses primarily on the cumulative effects of nonpoint sources of pollution, e.g. pollution from many diverse and diffused sources (see below). Watershed planning to control nonpoint pollution is one of 13 programmes initiated by the Puget Sound Water Quality Authority (PSWQA) in the state of Washington, USA, and has received national recognition in the United States as an innovative and effective model for addressing nonpoint pollution (Hansen 1990).

[1]Jim Lichatowich (pers. comm.), the former Assistant Chief of Fisheries of the Oregon Department of Fish and Wildlife, noted that at least until 1988 when he left the Department, the budget of a single Oregon state hatchery was greater than the habitat protection budget for the entire eastern half of the state.

The explanation for the success of this planning rests on two levels of analysis. The first level contrasts the new watershed-based planning and implementation with past state planning and implementation, seeing the former as a potential improvement over the latter. At this level of analysis, a general explanation is offered as to why watershed-based planning has the potential to be more successful than centralized state planning in dealing with nonpoint pollution. I examine how the local plan became binding on all the central agencies and how it reinforced and extended the capacity of the fisheries management agency to protect habitat effectively by building a local constituency to support habitat protection. This constituency both insured that the local plan would be implemented and also assisted in habitat protection activities at the local level. The central thesis at this level of analysis is that, although protective legislation is a necessary condition for successful protection, it is an insufficient condition without organized local community activism.[2]

The second level of analysis concerns the specific conditions which permit this potential to be realized. Highly successful locally based water quality planning is contrasted with less successful cases in order to draw out the necessary conditions at the local level for successful watershed planning.

The successful cases are important for more than the mechanisms of effective habitat protection they illustrate. Just as significantly, they epitomize the increased public demand for participation in management decision-making and the changing constituencies of management agencies (Lichatowich 1990a). The traditional commercial and recreational stakeholders and users of fish, wildlife, and even forest resources are no longer the only constituencies with whom policy must be discussed. A much broader public is now concerned. People want habitat protection not simply in order to have more fish, but also in order to enhance other values, such as water quality, sustainable ecosystems, disappearing wildlife, and quality of life. Management agencies no longer have a monopoly on setting management goals, as the public has taken a stronger leadership position and is prepared to decide which management risks are acceptable (Kellert 1980; McEvoy 1986; Lichatowich 1990a, 1990b; Rees 1990). Furthermore, on the local watershed level, participation in planning allows local knowledge and concerns about the watershed ecosystem to be articulated as community values and choices in management decisions. The community focus of public leadership in habitat protection can be a particularly effective way of organizing and mobilizing public opinion and action on habitat protection.

After briefly presenting the genesis of recent government policy on habitat protection in Washington State, I analyse the elements of habitat protection planning in Sequim Bay, Clallam County, where a highly successful and supported watershed plan is being implemented. I compare the planning process in Sequim Bay with elements of five other successful cases and contrast them with elements of the planning process in three other areas which were less successful. I considered watershed plans "less successful" when they did not involve a broad spectrum of watershed residents, nor mobilized a local constituency which became educated about nonpoint pollution issues and energetic in pressing for remedies. These represented two thirds of the 12 "early action" watershed plans initiated in Washington in 1987–88.

Background of State Policy on Fish Habitat Protection

Effective government policy on habitat protection in Washington State originated in a general sense from the 1980 Phase II decision of U.S. v. Washington ("the Boldt decision"). The 1974 Phase I decision ruled that the treaties signed by western Washington tribes guaranteed them the opportunity to catch 50% of the salmon runs passing their "usual and accustomed" fishing places, i.e. their traditional fishing places and usual fishing stations. The Phase II 1980 judgment reasoned that the first right was meaningless unless the tribes also had the right to protect the habitat on which the salmon depended to spawn and rear: freshwater streams, bays, and estuaries. Although the exact force of the Phase II decision has since become unclear because of technicalities (see Cohen 1986), it precipitated a series of irreversible events. One of these was the 1987 Timber, Fish, and Wildlife (TFW) Agreement, which established both higher standards and a local participatory process for implementing habitat protection for fish and wildlife in riparian zones and in certain uplands. This agreement is periferally relevant to this discussion and is discussed elsewhere (Pinkerton 1990).

Most importantly for the present analysis, the original court decision recognized a general tribal right to protect fish habitat. This right has since been expressed in several ways: (1) tribal representation on the PSWQA board (described below) and on the Forest Practices Board, which regulates fish and wildlife protection from logging and silvaculture, (2) funding for six tribal water quality biologists to work in Puget Sound,[3] (3) mandatory tribal participation on a policy and technical level in local watershed planning processes, and (4) tribal court cases against the Washington Department of Ecology, when the later was alleged to be falling short of its mandate to protect water quality. In general, the tribal right to protect fish habitat has been a catalyst for tribal alliances with environmental and other public interest groups and for the generation of greater public pressure on state agencies to provide greater habitat protection. The formation of the PSWQA is one response to the focusing of greater public pressure on environmental protection.

Puget Sound Water Quality Authority

In Washington State, industrial and household impacts on water quality in salmon streams and shellfish beds are regulated by standards set by the Department of Ecology under the Water Pollution Control Act and also by the state Departments of Fisheries, Wildlife, Natural Resources, Health, and Parks under other laws, including the State Environment Policy Act (SEPA). Federal laws and agencies also play a role. However, in the early 1980s, industrial and real estate development was outstripping the ability of these agencies to play a significant role in water quality protection. The populations of some counties in western Washington were growing at a rate of 20%: 20 000 to 30 0000 new homes per year (1988 figure).

Washington Department of Fisheries' (WDF) inability to deal with these developments is illustrated in the following example.

[2]The gains in effectiveness of policies through local participation in their development and implementation are discussed in more detail in Pinkerton (1989) as part of the development of middle-range theoretical propositions about fisheries comanagement.

[3]Through the Centennial Clean Water Fund, created by an $0.08 per pack tax on tobacco products, the Department of Ecology funded six regional positions to allow treaty tribes in the state to participate in water quality planning.

The habitat protection division of the WDF had only one full-time staff person for the area of 3500 km^2 surrounding the Sequim Bay watershed planning project to be described below. One person cannot adequately monitor all the ongoing and past habitat damage in this area, review the logging permit applications (approximately 600 a year), water flow alteration permit applications (approximately 240 a year), and water rights applications (approximately 25 a year) for potential habitat damage, as well as participate in the lengthy meetings and field reviews associated with the TFW process. In addition to inadequate staffing, there was no coordinated planning among agencies involved in water quality protection. While the pollution from one industry might be at an acceptable level when viewed in isolation by one agency, the cumulative effects of *all* sources of pollution on one watershed might be unacceptable. Regulation loopholes such as the single family dwelling exemption under the Shorelines Management Act compounded the problem.[4] In addition, curent regulations were not enforced in many cases. Environmentalists believed that development pressure on the local regulatory process (e.g. for building permits and logging permits) was so great that only an organized and coordinated program could successfully mobilize and focus the political will to effectively protect habitat.

The worsening of water quality in Puget Sound, and the lack of effective institutional arrangements to deal with this problem, was a major concern of the environmental groups which organized the Puget Sound Alliance in 1984. Through their efforts, the PSWQA was set up and funded by 1986 and produced its first water quality management plan in 1987. The PSWQA's plan, which is periodically revised, proposes specific remedial actions; the PSWQA then works with various existing agencies to produce budgets for carrying out the actions, using these agencies' staff. In some cases, the plan's budget permits additional staff to be hired by these other agencies: four water quality staff have been added to the Department of Ecology for the nonpoint pollution programme alone through this process. The other agencies are mandated to work with the PSWQA in carrying out the plan.[5] The PSWQA thus acts as the comprehensive envisioner of overall solutions and the instigator of particular programs, and sometimes projects, for carrying them out. Once initiated, these projects are generally assigned to other state agencies or local government, which manage funding for planning and implementation.

As part of its 1987 plan, the PSWQA launched a major new initiative to address "nonpoint source" pollution, i.e. pollution from diverse sources such as animal waste or pesticide runoffs from farms into streams. Nonpoint pollution is more difficult to analyze and regulate because the effluents do not appear in one specific place (out of the end of a pipe) or at one specific time. In addition, PSWQA's nonpoint program addressed the need to understand the combined and cumulative effects of pollution from different sources within a particular watershed and to take a basin-wide approach to water quality protection. Instead of trying to regulate each pollution source separately through a separate agency, the nonpoint programme aimed to develop a local watershed water quality plan which analyzed and addressed all forms of pollution comprehensively. Affected agencies were then asked to support the plan and participate in its implementation, coordinated by the lead agency, usually the county.

A key aspect of PSWQA's conception of successful water quality planning on a watershed level is public involvement in creation of the plan and in education of citizens of the watershed. Public involvement thus created the political will to implement the plan through public pressure on agencies. Just as important, it created the political will and widespred local involvement necessary to get significant compliance and support for life styles which are less destructive to water quality.

The PSWQA's regulation governing the watershed planning process — Chapter 400-12, Washington Administrative Code (WAC) (State of Washington 1988) — went into effect April 1988. This regulation called for counties to rank watersheds of greatest concern and to "solicit meaningful and substantive participation by the general public and affected parties..." Before the regulation was completed, however, the PSWQA selected six "early action" watersheds which were identified as requiring immediate attention. Six additional early action watersheds were selected through competitive applications to the Department of Ecology for funding to complete the first round of community-based planning. Department of Ecology staff provided technical and advisory assistance during the planning process. After the county, watershed community, Department of Ecology, and other review agencies gave final approval of the plan, the county applied to the Department of Ecology for funding to take the actions called for in the plan. The next section analyzes how the process operated successfully by focusing on one such community planning exercize.

Sequim Bay Watershed Management Plan

The Sequim Bay watershed near Port Angeles in Clallam County on the Olympic Peninsula was chosen by the PSWQA as one of 12 special "early action" watersheds for planning because previous studies already showed the need for imediate attention to water quality problems. In the case of Sequim Bay, its rich shellfish beds and fisheries were at risk from increasing levels of pollution. A new marina, livestock use of streams, failing septic tanks, erosion from timber felling and transport, and steady population growth all promised to worsen the problem unless addressed. The county was encouraged to apply for funding and hired a coordinator to convene an all-citizens committee including all concerned parties in the Sequim Bay watershed. Their role was to plan the clean up and prevention of unacceptable levels of pollution in the Sequim Bay watershed. The 22 members of the Watershed Management Committee included farmers, commercial shellfishermen, sportfishermen, the Jamestown Klallam tribe, environmentalists, boaters, realtors, local business, local government officials, and port officials from nearby Port Angeles.

Up to the point of selecting the potential early action areas, state policy and state-level initiatives were obviously key to the water quality planning process. As previously stated, state policies and initiatives were necessary preconditions to effective community planning. The point of this account, however, is to show that state initiatives alone are not enough. The state can threaten to set arbitrarily higher water quality standards, or it can attempt to enforce existing standards despite community

[4]Regulations regarding stormwater management systems did not apply to the piecemeal regulation of single family homes. Yet a large number of these homes along the shoreline have an unacceptable cumulative effect.

[5]In February 1990, Engrossed Substitute Bill 2482, reenacting and amending the Puget Sound Water Quality Act, Chapter 90.70 RCW, was passed by the state legislature. The legislative changes focussed on point pollution and have not affected the nonpoint pollution program which is the subject of this discussion.

will. To some degree, watershed planning did occur with a concern that "the state may do it if we don't do it first." It will be obvious from the following account, however, that a state-imposed plan could not possibly have garnered the support and volunteer effort which resulted from the community planning process. It is community "ownership" of a project through a consensus planning process which makes local planning effective as a habitat protection instrument.

Watershed planning in Sequim Bay was complex, however. Residents of Clallam County were known as the front-ranking financial contributors to organizations opposed to Indian treaty rights. The Sequim watershed was formerly an agricultural community, but now, 45% of its 5445 residents are over age 55. The population is turning increasingly to retirement and tourism. These local groups had never met with a tribe, and most had never met with each other. Some observers predicted a planning process would not last three months.

Principles for Effective Community-Based Watershed Planning

The analysis which follows drew generalizations from comparing the Sequim Bay process with five of the other nine successful projects and contrasted the successful processes with three which were acknowledged by all interviewees as less successful. I interviewed key participants in all planning processes, in the PSWQA's nonpoint programme, in the Department of Ecology's nonpoint programme, as well as some county officials and coordinators. I observed the Sequim Bay planning meetings at several points and interviewed the coordinator repeatedly over the two-year period of planning, plan review, and plan approval. A set of principles for effective community-based planning was generated, and each is discussed below.

(1) *The local lead agency must have a clear sense of purpose and the authority to assure implementation of plan recommendations*. In all but two cases, the county was the lead agency and had a clear intention to benefit from available funding by initiating a viable local planning process with outside professional help. In two less successful cases where the county was not the lead agency, the local agency which applied for the planning grant did not have a strong commitment to the local planning process, but was persuaded to attempt the exercise by a closely allied federal agency, which facilitated the planning grant application. The local agency already possessed a study of local water quality problems and viewed the planning process as an extra "frill" between the study and implementation of the study recommendations. As officials were reluctant to engage a professional planner or trained coordinator for this process of perceived lesser importance, the local agency used its own staff. Many potential supporters of and participants in the planning process abandoned the process at an early stage; time and credibility were lost before the Department of Ecology felt forced to require that more experienced staff be hired. Eventually a professional plan was written for both watersheds, but with minimal community education and involvement in the process. Although both plans will probably be implemented to some extent, they will not enjoy the degree of support of the more successful plans whose perceived legitimacy and authority guarantees them a high level of participation and compliance. In these two cases, the local agency may feel it has been successful, but has forfeited the possibility of a much greater success.

(2) *The coordinator of the planning process must be highly experienced in interest-based planning*. The most successful projects had coordinators who were also experienced in the issues. The PSWQA has now produced a handbook (Gordon 1989) detailing the most effective ways to guide the group process and has provided some training for coordinators. However, these aids should be considered supplements rather than substitutes for experience. In particular, skills in providing private caucuses, establishing a sense of fairness, and building consensus are critical (see below). A qualified coordinator is the single most important factor in the success of a planning process. In all three less successful plans, the lack of a coordinator with previous training and experience in group process was a key factor. One remarkable successful case managed without a qualified coordinator because two group members representing opposite perspective alternated the role and had enough experience between them to keep a fair process going. This should be seen as a lucky exception to the rule.

(3) *The selection of watershed committee members must include a balance of representatives from all the affected local interests*. The negative way of stating this rule is that any group with the power and influence to significantly damage community support must be included. In most successful cases, the planning coordinator and/or other county staff selected the committee members. In some cases, the county commissioner added another member. The balance was such that no single sector could have a majority, but would have to work with other sectors. The selection process was advertized in the local media, by mailings, and in some cases by directly soliciting representatives from key affected organizations. Volunteers for the watershed committee filled out a form or wrote a letter explaining their interest, whom they believed they represented, and the nature of their commitment to the process. The letters were screened and, in some cases, candidates interviewed. During interviews, the coordinator explained the consensus decision-making rule (see below) and tested the candidate for open-mindedness or willingness to consider a variety of options. The candidate was also questioned about his/her commitment of time and energy to the process of attending meetings regularly for over a year. In some cases, attendance of meetings was taken, and two consecutive absences disqualified a committee member. Such rules reflected the quantity and seriousness of the information committee members were asked to absorb. In other cases, the coordinator generated so much interest and enthusiasm for the project that attendance remained high without rules. A committee member's previous experience in a community planning process was also considered valuable, and at least some candidates were selected for this reason.

The need to keep this balanced mix of representatives working together further underlines the need for an experienced coordinator. Two of the less successful watershed committees started with a talented and experienced mix of members who were very excited about the idea of bringing salmon back to their watersheds. However, most of them ceased coming after the first three meetings because the inexperienced coordinator "talked down" to committee members in an attempt to educate them and did not know how to involve them in the process. The final product of the committee therefore had a much smaller base of support and more limited credibility. The third less successful committee was located in a sparsely populated area and had few experienced committee members to draw upon. This increased the requirement for a highly qualified and experi-

enced coordinator. With both positive factors absent in this case, the committee had a lower chance for success.

(4) *Technical and educational resources must be available to the coordinator and must be used judiciously by the coordinator*. The experienced coordinators spent a great deal of time on education during at least the first two or three months of the process. In some cases a technical committee, composed of the professionals from the constitutent organizations, met separately and helped develop a series of educational presentations for the watershed committee. The Department of Ecology also made materials and technical assistance available, although some of these were still in preparation when the early action projects were beginning to meet. The experienced coordinators gathered, organized, and presented educational materials in a series of discussions of the water quality problems of the particular watershed. Some coordinators used an early educational meeting as a workshop on group process. Guest speaker experts on both process and substantive issues were also effective.

In the three less successful processes, the inexperienced coordinators either spent too much time attempting to educate the committee without interactive techniques ("an uninterrupted monologue") or they did not sufficiently assemble and present available educational material. While education is key, it must be combined with open discussion of the problems and concerns of local residents. Watershed committee members must become active participants in the educational process.

(5) *The coordinator must provide guidance to the watershed committee in how to set and reach long-term goals*. The coordinator must be able to present the educational materials described above while eliciting the concerns of the different community interests. All interest groups should have a clear understanding of the others' needs and goals. In other words, the problem of how the community was (or soon would be) in violation of state water quality standards was set in the context of increased understanding and respect for the needs of different interest groups. The successful coordinators presented the process as an opportunity for the community to solve the problem itself, rather than leaving open the possibility that government might eventually impose a "solution" they would find less acceptable. Through a process of consensus-building (see below), the coordinator led the committee to define a common goal or set of goals and developed a strategy and action plan for achieving those goals. In the process of defining the level of water quality protection that the community wanted, and the steps that must be taken to achieve that level, the committee was gradually led to consider and evaluate a range of options for achieving their goals.

Key to this process was the way the problem of pollution was defined. Skilled coordinators were able to get the committee members to focus on the solution (the goal) rather than on who was causing the problem. This enabled a more objective view of how all committee members and all sectors of the community could contribute to the solution. It also encouraged good will and voluntary compliance on the part of polluters. On one creek in the Sequim Bay watershed, for example, the five most important cattle farmers who created 90% of the bacteria in the creek adopted best management practices to reduce or eliminate runnoff from their pastures into fish streams. One year later, there is already a 10% reduction in fecal coliform levels. County officials also adopted a new policy on septic system surveys after only 12 weeks of meetings. In contrast, in one less successful planning process, committee understanding of the problem and committee identification of common goals did not occur. As a result, one farmer was denying even at the end of the planning process that his cows were polluting the stream, even though it was clear that they were.

(6) *The coordinator must help build consensus among committee members*. Most planning committees started with a collection of interests which were either polarized or potentially polarized. The coordinator of the Sequim Bay planning process drew upon her experience working with industrialists and tribes who had agreed to cooperate in order to stay out of court after the Phase II U.S. v. Washington decision. She had also been a mediator with the TFW Agreement negotiations. This experience led her to develop incremental steps in cooperation through a series of cooperative projects in which team members participated throughout the planning process. These cooperative projects often included other community members and became part of educating the community at large and creating a constituency in the community which supported water quality planning. They also helped committee members get out of formal public roles and recognize their ability to work together toward committee goals.

Cooperative projects in the Sequim Bay process included participation by 100 citizens in marine debris clean-up on five beaches, a day dedicated to the collection and safe disposal of toxic chemicals, and joint application by the tribe and the farmers to stabilize a major landslide which threatened to continue eroding into a creek. Volunteers and landowners also jointly rehabilitated a half-mile of creek through construction of meanders and restoration of bank vegetation; they constructed five spawning pools in another creek through the joint efforts of the tribe and a local sportsmen's group, with assistance from WDF. After this last project, a sportsman who had formerly worked against the tribes stated: "We see now that we have more in common than we thought we did, and we're all looking down the same tunnel and we all hope the same light is at the end: more fish for all of us." The act of working together on specific projects to accomplish a common purpose is a useful tool for building consensus. It creates a sense of a shared goal and focuses people not on placing blame but on developing solutions.

Another method of focusing on solutions rather than blame was to identify the pollution by type rather than source. The Sequim Bay coordinator divided pollution into "sediments" and "bacteria," for example. Since bacteria come from both people and cows, the category avoids singling out one polluter. This method of describing pollution is now reflected in the revised regulation.

Another method of developing consensus was to explore issues in smaller groups or individually, either by doing some small group work during meetings or by encouraging people to visit the office outside of meetings. Outside caucusing has always been an important mediation tool, as it provides an opportunity for quieter group members to express their deepest fears and concerns to the coordinator. The coordinator can then discuss the issue in depth and help the concerned party to articulate it more clearly. The issue can then be aired with the group. This process helped committee members get to the point of expressing their real needs, rather than their "positions" (Fisher and Ury 1981). A publicly stated position does not always help a group arrive at a solution because it often does not assist in an understanding of what is really behind the position.

Once the members recognized a physical illustration of the possibility and the benefits of cooperation, and better understood different parties' needs, it became possible to think of

solutions which could incorporate the needs of all parties. This allowed people to stop focusing on each other and begin collaborating on mutual success. Such a solution then had a good possibility of having high credibility and of winning broad support in the community.

(7) *Community support for the plan will be strongest where a local constituency is built through community education and participation in volunteer projects.* Another factor in the strong community support for the Sequim Bay plan was the degree of community participation. Volunteer efforts included 350 youths who raised salmon in the school and later released them, 60 teachers involved in water quality education inside and outside the classroom, and six major stream projects, including rechanneling of two streams to avoid major pollution. Other volunteers included "Baywatchers," a core group of watershed residents who took a nine-week course on water quality issues and then performed a variety of community service projects. This group then became a resource for the rest of the community (see Hansen et al. 1990). This concept, based on the 1930s "Master Gardener" model used by the U.S. Department of Agriculture Co-operative Extension to teach food growing and preservation, was pioneered in Sequim Bay for water quality, has now been adopted by four other counties, and is being considered as a model nationally. The Baywatchers became the core of the local community constituency which supported the plan, helped educate the community, and will apply political pressure to fully implement the plan.

(8) *The plan developed by the committee must include a strategy for improving water quality, expressed in a series of recommendations which lead to an action plan.* The recommendations produced by the Sequim Bay watershed committee emphasized education, technical assistance, peer pressure, and an opportunity for voluntary compliance, before the use of enforcement. The following example illustrates a standard procedure followed in the plan. If a complaint about farm practices polluting a stream is initiated by or filed with the county or Department of Ecology, the complaint is forwarded to the local farmers' voluntary association, which contacts the landowner and offers technical assistance to remedy the situation. Technical assistance can include cost sharing of improvements. A recommendation for enforcement is sent to the state only if the landowner is unwilling to cooperate.

In addition to 11 recommendations for education-related actions, the Sequim plan recommended 28 actions related to keeping bacteria out of the water from agricultural sources, septic system sources, and marine sources. Nineteen recommended actions were produced for keeping sediment out of the water from landslides, forestry sources, culverts, inadequate stream corridors, log yards, grading and clearing, and stormwater drains. Eleven recommendations were made for actions to keep toxics, chemicals, and plastics out of the water and five recommendations on how to improve and enhance habitat. The Sequim Bay plan was the first in the state to specifically target fisheries habitat protection in recommendations for its watershed. A specific habitat protection objective was considered for inclusion in the 1991 PSWQA plan. Agency review of the preliminary draft plan produced an impressive map of interagency initiatives and responsibilities and a schedule for implementation and ongoing monitoring of each recommended action (Baril et al. 1989).

The plan strongly advised creation of new ordinances to control grading and filling and to allow inspection of septic systems by county officials. Just as important, the plan recommended that certain current laws be enforced properly at the state and county levels and that adequate penalties be attached to noncompliance. These included the proper enforcement of regulation concerning point pollution from the Sequim sewage treatment plant and enforcement of logging road building and maintenance standards. The significance of these recommendations is that they focus public attention on the problem and force agencies to work out ways — including securing more funding or cooperation from other agencies — to address them. The plan in fact specified which agencies were responsible for responding to each recommendation.

Watershed planning also allows agencies and citizens to identify site-specific concerns which need more effective responses than mandated by agency regulations (unless existing law is preemptive). It also builds constituency support for the enforcement of current state guidelines. For example, the Sequim plan goes farther than the TFW requirements and recommends a 25-foot buffer on all Type 4 (tertiary) streams in the watershed. These buffers are negotiable on a site-specific basis for Type 4 streams in the TFW Agreement. In addition, an innovative recommendation goes beyond the terms of reference of the TFW Agreement: that approval of cutting permits be based on consideration of cumulative impacts.[6] In other words, each logging permit cannot be considered in isolation, but in terms of how it, in conjunction with previous logging in the same area (and, by implication, future plans for the entire area), will affect water quality. The recommendation is tantamount to the community being able to comment on the size of clearcuts in its watershed, a consideration which the timber industry insisted could not come under negotiation in the TFW Agreement. Ironically, the makers of the plan have forced the county, which was excluded from the original TFW agreement, to take a front seat in insisting that timber interests be responsible for their impact on other public resources. In the Sequim watershed, water quality issues have proved to be a unifying force which puts issues such as timber harvesting in a new perspective.

(9) *The agency overseeing the planning grant should be willing to intervene in a project which is not proceeding successfully, but should avoid creating rigidities.* The community planning concept requires senior levels of government to take an oversight and supportive stance once the project is approved and funded. In the case of the three less successful early action projects, however, hindsight has made many observers believe that the ill feeling over government intervention would have been preferable to the ill feeling and alienation left in the communities from the less successful process. As one supervisor sees it: "It may be a decade before that community will be willing to try anything like this again."

State intervention was probably desirable in at least two projects because of the lack of qualifications of the coordinator. One weakness in the process was that the local agency hired

[6]In practice, the TFW cooperators have not been able to avoid considering cumulative impacts. The public concern with this issue finally forced the Department of Natural Resources (which regulates logging practices) to reverse an earlier intention to log Gibbs Lake on the Olympic Peninsula (see Pinkerton 1990), to take seriously the opposition to logging around Lake Roesiger in Snohomish County (see Halbert and Lee 1990), and to finally convene a Sustainable Forestry Roundtable to consider cumulative effects and rate of harvest. It is significant to note that the problem of cumulative effects was being addressed in the Sequim Bay plan in January 1989, about seven months before these other crises.

the coordinator with no guidelines or minimum qualifications required. The problem could have been corrected by simply requiring a sufficient level of experience in this position. Closing this loophole would have addressed vitually all the serious problems which emerged in the early action projects.

Unfortunately, government agencies are easily tempted to avoid the problems of interference with poor coordinators by writing much more rigid guidelines for conducting the planning process. One of the strengths of the community-level process was the flexibility to develop different ways to conduct the process. The regulation allows an experienced coordinator the freedom to adapt to local ideology and circumstance and discover the most effective methods of guiding the process and mobilizing interest and support. Greater rigidities introduced into the process may increase the problem of antigovernment feeling which already makes some processes more difficult. A brief application of this principle to government reaction to the Sequim Bay plan will illustrate the advantages of flexibility.

During review of the plan in spring 1989, the Department of Ecology expressed concern that there were no specific plans to limit population growth and land use through zoning ordinances. Sequim committee members insisted that they disliked the rigidities of zoning ordinances and that they had other ways of dealing with the problems. There appeared to be a consensus in the community that alternatives to zoning were feasible, although they would require more participation and funding. Rigid application of zoning ordinances in Clallam County in the past had resulted in a rush to subdivide for denser housing and to harvest timber, producing habitat loss. The Department of Ecology has offered funds for two more years to implement the plan recommendations, and a 20% matching contribution from the county (through taxes or assessment) has been supported for longer-term monitoring and community involvement. Because the Department of Ecology was eventually willing to accept the community method, rather than imposing its own, strong community support of the plan was possible.

Synthesis and Conclusions

In rural or transition areas such as Sequim Bay, habitat protection efforts by state agencies will be largely overcome by development pressure unless a local planning and implementation process can mobilize and focus local political will and activism. This paper has examined how a state program empowered local communities to participate in habitat protection and why this was far more effective than state planning and regulation alone. The creation of legitimacy, accountability, and a sense of control were the key factors in mobilizing the local community to take a large role in habitat protection. Below I briefly synthesize and restate the argument presented.

Development of the Sequim Bay Watershed Management Plan, and other successful PSWQA early action plans, was a consultative process involving extensive community education, consensus, and constituency building. Local groups and agencies in these rural or transition areas were challenged to preserve and enhance endangered fisheries habitat, as well as other perceived "quality of life" values. In the process the community was able to consolidate, articulate, and put into action a set of values and priorities regarding habitat protection which won widespread support in the community.

The focusing of collective community will into a plan resembles the adaptive process by which small-scale, preindustrial (and, in some situations, modern) societies dependent on key resources adjust their behavior to conserve these resources (Durham 1976; Williams and Hunn 1982; Freeman and Carbyn 1988; Berkes 1989). The nature of resource "dependence" can be thought of in the modern context as a perceived requirement for quality of life or the continuance of a chosen livelihood. The dependence was given recognition by the willingness of the community to contribute financially to solutions. Even more important, the community generated these values and activities *because* of the perception that it has some real control of the situation: that it could actually protect its own watershed. A large body of research in psychology indicates that people are able to mobilize energy and perform optimally and rationally under conditions of perceived high "self-efficacy," or ability to exert effective control (e.g. Bandura 1982). This finding is consistent with ethnographic evidence that people are willing to contribute major volunteer effort when they feel they have control.

One result of the community's perceived need for control and involvement has been a community insistence that water quality be improved "our way," with an emphasis on education and site-specific considerations before standardized regulation and enforcement from outside. The benefit of this possibly higher-risk and more labor-intensive route has been that the Sequim plan enjoys widespread support from all sectors of the community, such that the county officials charged with implementation must take it seriously. Elected officials responded positively when they could be seen as supporting a popular agenda over specialized sectoral interests in logging, agriculture, or real estate. A 1989 poll of households in the watershed showed not only universal support, but a majority said "We haven't gone far enough yet." Ninety percent of the respondants supported more tax for implementation of plan recommendations. The county has set up a new Water Quality division to coordinate plan implementation and recommended grant funding for the next five years of further planning and implementation. This level of support, and the structure of accountability to the local lead agency (the county) and the local political constituency (the community), has created a situation far more favorable to the enforcement of existing regulations (currently unenforced) and of plan recommendations. Community-controlled planning and implementation creates a legitimacy and accountability which is more powerful than conventional state planning.

The most important insight emerging from these case studies is that the effectiveness of habitat protection can be enormously increased because of the way the local plan's legitimacy and accountability mechanisms combine with the mobilization of local volunteer efforts. With modest funding — one coordinator for the Sequim Bay planning process, seed money for projects — considerable volunteer effort can be generated and the major groundwork in habitat protection, enhancement, and rehabilitation can be carried out. The legitimacy created by the local production of a focussed and coordinated programme enables the generation of the political will to turn community recommendations into action. Regulations are now backed up at the community level, and government agencies are not allowed to respond to special interest group pressure: they are also accountable to the community plan. In this situation, government habitat biologists play an important role overseeing activities, applying standards, providing seed money, and carrying out their mandated role with support rather than interference. Government alone, however, is clearly unable to do all the work that is needed to protect habitat. The involvement of local

groups should be seen as an enormous asset for the responsible, long-term, sustainable protection of fish habitat.

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LETTERS AND COMMENTS
LETTRES ET COMMENTAIRES

Consumption, Growth, and Allometry: A Comment on Boisclair and Leggett (1989a, 1989b, 1989c, 1989d)

In a recent series of papers, Boisclair and Leggett (1989a, 1989b, 1989c, 1989d) used data from 12 lakes in Quebec to examine growth, consumption, and the energy budgeting process among yellow perch (*Perca flavescens*) populations. Their major conclusion was that observed differences in growth rates among populations were not related to differences in food consumption rates but were, instead, due to unmeasured differences in activity levels exhibited by fishes among lakes.

While we agree with Boisclair and Leggett that activity is one of the most poorly understood components of fish energy budgets, we feel that their conclusions are unsubstantiated due to errors in approach and analysis. We address three basic issues. First, the analysis of the relationship between food consumption and growth rates (Boisclair and Leggett 1989a) is inappropriate. This is crucial because all subsequent analyses by Boisclair and Leggett (1989b, 1989c, 1989d) were based on the lack of correlation between relative growth rate and relative consumption rate in Boisclair and Leggett (1989a). Second, Boisclair and Leggett's model of gastric evacuation contained a size-dependent bias which resulted in inaccurate estimates of food consumption for large and small fish (Boisclair and Leggett 1988, 1989a). Finally, Boisclair and Leggett (1989d) used a bioenergetics model inappropriately in estimating activity level of perch.

Growth Rate versus Food Consumption

Boisclair and Leggett (1989a) concluded that food consumption rates did not explain differences in growth rate because a regression of relative growth rate on relative daily ration for age II+ and age III+ fish was not significant. The relationship was significant and positive for age I+ fish, as was the relationship between absolute growth rate and absolute daily ration for age-classes I+ and II+.

Any analysis of the relationship between growth and consumption among fish populations must account for variation in body size, water temperature, energy density of both prey and predator, and the interactions among those factors. Boisclair and Leggett (1989a) attempted to account for body size, temperature, and caloric density of prey in their analyses; however, they did so inappropriately. As a result, the effects of these factors were not removed from their analyses.

Body size — The authors addressed body size differences among lakes by comparing relative rates of growth and consumption rather than absolute rates. In spite of this, their analysis remained biased by body size.

Consumption rates and respiration rates have been described as allometric functions of body size (aW^b) with weight coefficients (b) of less than 1 (Kitchell et al. 1977; Peters 1983). The conversion to relative rates divides these functions of weight by W^1 with the result that relative rates are allometric functions of weight with a negative coefficient (Peters 1983). These allometric functions of body size, in combination with nonlinear relationships between growth or consumption and temperature, can produce unexpected results when comparing growth and consumption among populations.

An analysis of the relationship between relative growth rate and relative consumption rate among different populations can show any pattern from a positive to a negative correlation depending upon factors discussed below. This is the case even though increased consumption in any given lake will produce increased growth. Boisclair and Leggett's (1989a) comparison based on relative rates would be an appropriate analytical approach only if physiological processes were proportional to W^1 (i.e. no allometry). This is perhaps nonintuitive due to the nature of allometric relationships (Peters 1983); therefore, we use an example to illustrate the effect of initial body weight on both absolute and relative measures of growth and consumption.

We used the bioenergetics model of Hewett and Johnson (1987) to calculate absolute and relative growth rates for yellow perch with initial weights from 10 to 30 g and for consumption rates between 35 and 55% of maximum consumption (chosen to fall within the ranges for age II fish in Boisclair and Leggett (1989a)). We used a typical temperature curve based on the data in Boisclair and Leggett (1989a). For all estimates the activity parameter was set to 1 as in Kitchell et al. (1977). Relative rates of growth and consumption (relative daily growth and relative daily ration) were calculated according to Boisclair and Leggett (1989a).

For each initial weight, absolute and relative growth rates increased with higher consumption level (Fig. 1 and 2). Note that as initial body size increases, absolute consumption and growth rates increase but relative rates of both growth and consumption decline (Figs 1 and 2). If conversion to relative rates did account for body size differences, then the lines in Fig. 2 would lie on top of one another. That is not the case. Points can be subsampled from the data in Fig. 1 and 2 that result in negative, positive, or insignificant relationships between relative growth rate and relative consumption rate.

For example, taking a series of points for fish varying in initial size in which larger fish are feeding at a higher proportion of maximum consumption, absolute growth rate and absolute consumption rate show a positive correlation (Fig. 1, sample points). When these same points are converted to relative rates of growth and consumption, they show a negative correlation (Fig. 2, sample points). Other initial weights and consumption rates sampled from these figures could produce any relationship between relative rates of growth and consumption. The lack of positive correlation between relative rate of growth and relative rate of consumption in Boisclair and Leggett's (1989a) analysis is not relevant to the issue of whether differences in consumption rate explain the observed differences in growth rate among perch populations.

Temperature — The effect of temperature differences between lakes on fish growth rates is not discernible using lin-

ear regression analysis. Temperature effects on growth and consumption interact in a nonlinear fashion with body weight throughout a given time period. Temperature effects cannot be dismissed by noting that seasonal mean water temperatures differed by only 3° (Boisclair and Leggett 1989a) or that there were no significant relationships between seasonal mean water temperature and the relative rates of consumption and/or growth. A difference of 3° in seasonal mean water temperature can be significant, as we will demonstrate. In addition, seasonal patterns are likely to be important; a warm spring and cool fall has a dramatically different effect on fish growth than a cool spring and a warm fall — differences which are not reflected in seasonal means.

We evaluated two temperature curves, using a bioenergetics model of perch growth, to determine the potential variance in consumption estimates that could be due to temperature in the analyses of Boisclair and Leggett (1989a). The two curves used were chosen from the upper and lower bounds on the observed temperatures in lakes examined by Boisclair and Leggett (1989a, fig. 4). The lower curve represented a seasonal mean water temperature of 18°C, and the upper represented a seasonal mean water temperature of 21°C (comparable with the observed range of 18.1–21.3°C; Boisclair and Leggett 1989a). Using initial and final weights, and the number of days in each growth interval from Boisclair and Leggett (1989a) for each lake/age-class combination, the mean difference between consumption estimates developed using the two temperature curves for each age-class (averaged across lakes) was calculated. These ranged from 17% for age I+ fish to 24% for age III+ fish (Table 1). This suggests that a significant proportion of the variation in consumption rates between lakes may, in fact, have a temperature basis.

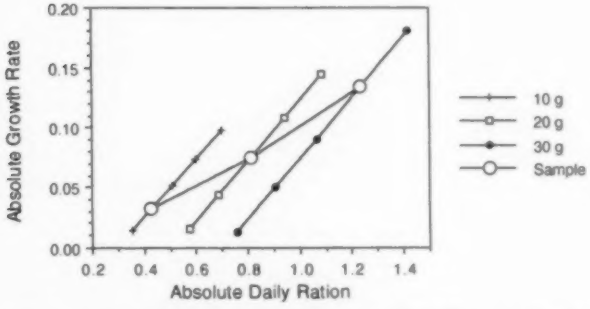

FIG. 1. Relationship between absolute growth rate and absolute daily ration for three initial sizes of perch (10, 20, and 30 g) and five rates of food consumption (0.35, 0.40, 0.45, 0.50, and 0.55 of maximum consumption rate). Initial sizes and feeding rates were chosen to fall within the range from Boisclair and Leggett (1989a). Absolute daily ration and absolute growth rate were calculated according to Boisclair and Leggett (1989a). Sample points show the effect of larger fish feeding at a higher proportion of maximum consumption rate.

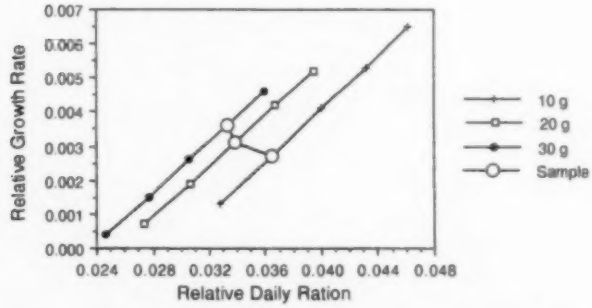

FIG. 2. Relationship between relative growth rate and relative daily ration for three initial sizes of perch (10, 20, and 30 g) and five rates of food consumption. Initial sizes and feeding rates were chosen to fall within the range of Boisclair and Leggett (1989a). Relative daily ration and relative growth rate were calculated according to Boisclair and Leggett (1989a). Sample points show the effect of larger fish feeding at a higher proportion of maximum consumption rate.

TABLE 1. Proportional increase in expected consumption due to change in seasonal mean water temperature from 18 to 21°C. Temperature curves from fig. 4 of Boisclair and Leggett (1989a). Beginning weight, final weight, and time interval from tables 1 and 2 of Boisclair and Leggett (1989a).

	Age group		
Lake	I	II	III
Brome	—	—	0.26
Bromont	0.20	0.25	0.27
Brompton	0.18	0.20	0.24
D'Argent	0.18	0.28	0.25
Drolet	0.14	—	0.24
Hertel	—	0.26	0.27
Magog	—	0.17	0.23
Massawippi	0.17	0.27	—
Memphremagog	0.15	0.19	0.22
Roxton	—	0.21	0.23
Silver	0.15	0.15	—
Waterloo	0.18	0.24	0.20
Mean increase	0.17	0.22	0.24

Caloric density — Energy budget studies for fish must take into account differences in energy density between predator and prey as well as seasonal or ontogenetic changes in energy density of both predator and prey (Stewart et al. 1983; Hewett and Stewart 1989). The effect of variation in energy density of prey among lakes or age-classes would likely be similar in magnitude to the effects of temperature discussed above and would contribute to the variation observed by Boisclair and Leggett (1989a). Typical prey items for yellow perch range between 500 and 1200 cal·g wet weight^{-1}, but the mean energy density of prey for different age-classes or populations would not be that extreme. Perch feeding on prey with lower energy density would show a lower growth rate than other perch with the same consumption rate that fed on prey of higher energy density.

Boisclair and Leggett (1989a) incorrectly reported no significant differences in prey energy density either among populations or among age-classes. Contrary to their conclusions, their analyses showed that significant variation in caloric density of stomach contents did occur among populations and age-classes. The analyses of variance showed all two-way interactions among the main factors (age-class, population, and date) to be significant, which means that the effects of the main factors were significant, irrespective of the significance level of the main factors alone (Sokal and Rohlf 1981, p. 338–339).

It is not clear from Boisclair and Leggett (1989d) whether energy density differences between perch and prey were accounted for in the bioenergetics model estimates of consumption. Their mean energy density for prey was 634 cal·g

wet weight^{-1} (Boisclair and Leggett 1989a). A representative value for yellow perch is between 1000 and 1300 cal·g wet weight^{-1} (Craig 1977). If Boisclair and Leggett (1989d) assumed equal energy densities for perch and prey in the bioenergetics-based estimates of consumption, those estimates may be low by 55–100%.

Comparison of consumption and growth rates — If the analytical approach taken by Boisclair and Leggett (1989a) is incorrect, what can be done to compare growth and consumption among fish populations? We feel that the general approach taken by Hayward and Margraf (1987) is the best available method for comparing growth and consumption between populations of fish, despite the objections of Boisclair and Leggett (1989a, 1990) and Hayward (1990). Hayward and Margraf (1987) estimated maintenance ration using a bioenergetics model and used the ratio of estimated ration to maintenance ration as a measure of relative prey consumption rates (or as a measure of prey availability to fish) in different thermal habitats. The use of bioenergetics modeling to calculate maintenance ration can correct for differences in thermal history, energy densities of predator and prey, body size, and the interactions among these factors. Either comparing absolute growth rate with the difference between actual and maintenance consumption or comparing relative growth rate with the ratio of actual consumption to maintenance consumption may be appropriate to correct for body size differences among age-classes or populations.

Model of Gastric Evacuation

Both temperature and body size are important variables that affect the evacuation rates of fish. The effect of temperature can be much larger than effect of weight in the estimation of daily ration, depending upon the ranges of both factors in the field. However, the relative importance of weight effects increases at lower body weights because of the allometric nature of the relationship.

Boisclair and Leggett (1988, 1989a) modeled gastric evacuation rate as an exponential function of temperature (Eggers 1977; Elliott and Persson 1978). This model ignored weight effects on evacuation rates and may have produced biased estimates of daily ration for fish that span a broad range of weights.

Evacuation rates have been found to be an exponential function of weight, with exponents ranging from 0.33 to 0.41 (Pandian 1967; Jobling et al. 1977; Grove et al. 1978; Flowerdew and Grove 1979; Jobling 1981; Mills et al. 1984). Other workers that have found body size to affect gastric evacuation rates include Swenson and Smith (1973) and Jones (1974).

To estimate the magnitude of the weight-dependent bias in the consumption rate estimates of Boisclair and Leggett (1989a), we chose 0.4 as a typical estimate of the exponential effect of weight upon evacuation rate. We used the ratio of $W^{0.4}$ for either extreme of the range of seasonal mean weights within lakes (5.3 and 34.8 g) to the overall seasonal mean weight across lakes (16.9 g) as a conservative estimate of the relative weight-dependent bias that could have occurred, given that perch weights on individual sampling dates would have been both smaller and larger than the overall seasonal means. An overestimate of gastric evacuation rate would result in an underestimate of total consumption, while an underestimate of gastric evacuation rate would result in an overestimate of total consumption.

The ratio of $W^{0.4}$ for the smallest age I seasonal mean weight to the overall mean was 0.63; for the largest of the age III mean weights the ratio was 1.34. Therefore, Boisclair and Leggett's (1989a) estimates of consumption could have been 37% low for small perch and 34% high for large perch.

The relative bias (a factor of 2.1; 1.39/0.63) is comparable with the variance observed in evacuation rate as a function of temperature (fig. 3 of Boisclair and Leggett 1988). Much of the variance in the estimates of evacuation rate in Boisclair and Leggett (1988) could have been due to size effects. Boisclair and Leggett's (1988) model cannot accurately estimate consumption rates in the field unless some correction is made for the effects of body size on evacuation rates.

It is instructive to note that the method of estimating consumption used by Boisclair and Leggett (1988, 1989a) is a mathematical model based on physiological measurements, as are the methods of Kerr (1982) and Kitchell et al. (1977). Therefore, Boisclair and Leggett (1989d) compared three different model estimates of consumption rates, not "actual" consumption rates with two model estimates of consumption rates. Correction of the weight-dependent bias in the gastric evacuation model of Boisclair and Leggett (1988) would modify the consumption estimates of Boisclair and Leggett (1989a), making them more similar to the estimates based on the Kitchell et al. (1977) bioenergetics model.

Activity Level

Boisclair and Leggett (1989d) recognized that the bioenergetics model of Kitchell et al. (1977) could be used to calculate activity level. However, the method by which they used the bioenergetics equations to calculate activity was incorrect and resulted in a substantial overestimation of activity level.

Boisclair and Leggett (1989d) apparently solved for activity by substituting seasonal mean values for weight, temperature, growth, and consumption into the Kitchell et al. (1977) equation. These activity estimates were relatively high (mean of 1.8 × standard metabolism) and quite variable, including both uninterpretable negative activity levels and extremely high values.

Using seasonal mean values does not account for the nonlinear effects and interactions of weight and temperature on metabolism. One of the primary reasons for developing computer-based bioenergetics models is that growth is a Markovian process in which the interaction between temperature and weight is continually changing. The computer models of Kitchell et al. (1977) and Hewett and Johnson (1987) operate on a daily time step and then sum values over the interval modeled, accounting for the nonlinearities occurring over time. Estimates obtained using this approach may differ from those obtained by merely solving an energy balance equation with seasonal mean values.

We cannot precisely calculate the activity level required to fit the data of Boisclair and Leggett (1989a, 1989d) without temperature and energy density data for each lake. However, for illustrative purposes only, we created a temperature curve from the data in Boisclair and Leggett (1989a, fig. 4) that matches the seasonal mean water temperature reported for Brome, 19.8°C. We assumed 634 cal·g^{-1} for prey and 1200 cal·g^{-1} for perch. For age-class III from Brome, we estimated the correct activity parameter to be 2.5 which is far below the 3.88 estimated by Boisclair and Leggett (1989d), but which required near-maximum rations (94% of the maximum con-

sumption rate). Similar results for other lakes would substantially reduce the mean activity level in Boisclair and Leggett's data.

Conclusions

There is a strong need to develop better methods of making interpopulation (or age-class) comparisons of growth and consumption. Such methods need to account for differences in body size, thermal history, and energy density for both predators and prey. Proper techniques for such comparisons would give fisheries managers better tools to explore the differences in growth rates between fish populations. The analysis used by Boisclair and Leggett (1989a) does not appropriately address the question of whether growth rate differences between lakes can be explained by patterns of food consumption.

The methods of Hayward and Margraf (1987) provide a useful approach for comparing consumption and growth among populations. Using an index of actual consumption to maintenance consumption does, in part, correct for the effects of body size, thermal history, and energy density differences because the effects of those factors are included in the calculations of maintenance ration.

There are many ways in which a combination of data collection and analysis along with a bioenergetics modeling approach can be used to tease apart the relationships among the complex suite of variables that affect fish growth. Modeling allows the researcher to ask "What if" types of questions that can enhance one's understanding of growth processes. Boisclair and Leggett's (1989a) approach was to ask whether the growth rate differences between populations could be explained by differences in food consumption rates. It is, perhaps, more appropriate to determine what proportion of the observed variance in growth rate can be explained by food consumption, thermal history, initial body weight, or level of activity.

Growth in fishes is a complex process which is best understood from a multivariate framework. Examining growth from the viewpoint of single factors ignores the larger picture and excludes the interactions among factors which make growth a dynamic and interesting process. The effect of differences in consumption rate on growth rate in the analyses of Boisclair and Leggett (1989a) remains unresolved — Steven W. Hewett, *Chesapeake Biological Laboratory, Center for Estuarine and Environmental Studies, University of Maryland, P.O. Box 38, Solomons, MD 20688, USA*, Clifford E. Kraft, *Sea Grant Office, University of Wisconsin-Green Bay, Green Bay, WI 54311, USA*, and Barry L. Johnson, *Center for Limnology, University of Wisconsin-Madison, Madison, WI 53706, USA, and U.S. Fish and Wildlife Service, National Fisheries Research Center, P.O. Box 818, La Crosse, WI 54602, USA.*
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If Computers Could Swim or Fish Could be Programmed: A Response to Comments by Hewett et al. (1991)

We recently assessed the relative importance of the quantity of food consumed, the type of prey in the diet (taxonomic

groups and size classes), and a suite fish community descriptors (numerical density and biomass of fish species) in explaining among-population variability in fish growth rates of 12 yellow perch (*Perca flavescens*) populations (Boisclair and Leggett 1989a, 1989b, 1989c, 1989d).

Our analyses suggested that the percent contribution of specific prey taxonomic groups to fish diet together with fish community descriptors explained a greater proportion of the observed among-population variability in fish growth rates than did the quantity of food consumed (Boisclair and Leggett 1989a, 1989b, 1989c). In an important extension of our work, we estimated the magnitude and variability of fish activity rates using the Kitchell et al. (1977) bioenergetics model. We further evaluated the influence of assumptions designed to account for activity costs when food consumption is predicted using the Hewett and Johnson (1987) bioenergetics model (Boisclair and Leggett 1989d). Our work suggested that (a) fish activity costs can represent a large fraction of their energy budget (maximum ranging from 25 to 40% of the energy ingested depending on age classes), (b) fish activity costs may vary substantially among fish populations (minimum values not significantly different from 0% of the energy ingested), and (c) the assumptions relative to activity costs may result in a threefold difference between observed consumption rates and feeding levels predicted from bioenergetics models (Boisclair and Leggett 1989d).

The questions raised by Hewett et al. (1991) in their comment on these papers stem from three different, but equally important points: (1) their misunderstanding of some aspects of our methodology, (2) their inaccurate interpretation of our results, and (3) their assumption that, in their present form, bioenergetics models can adequately represent fish energetics. Our reply will therefore focus on the clarification of these points.

Growth Rate versus Food Consumption

Contrarily to the perspective presented by Hewett et al. (1991), we do not believe that our finding that the quantity of food consumed by fish played a secondary role in explaining among-population variability in fish growth rate should be interpreted as "food consumption does not have anything to do with growth." In fact, many of the relationships we presented in our series of papers suggested that the quantity of food consumed by fish contributed significantly to explaining the observed among-population variability in fish growth rates (Boisclair and Leggett 1989a, 1989b, 1989c). Furthermore, it is worth noting that the coefficients associated with food consumption were always positive. Finally, our multivariate analyses indicated that the quantity of food consumed by fish explained approximately 30% of the among-population variability in fish growth rates for the 12 populations we studied (Boisclair and Leggett 1989c).

We concluded: "Taken together, our work (Boisclair and Leggett 1989a, b, c, d) should be perceived, *not* as a rejection of the relationship between fish growth and consumption rates, but as an important reminder that other factors, some of which are potentially more important than consumption rates, can strongly interfere with this intuitively pleasing relationship." (Boisclair and Leggett 1990). This comment also applies to the many other factors known to influence fish growth rates. Taken individually, while all other variables are held constant, body size, water temperature, caloric density of prey, water acidity level, etc., certainly can and do influence fish growth rates. However, fish populations differ from computer models in that they rarely (if ever) experience changes in a single variable while every other aspect of the environment remains constant. Furthermore, we can produce model predictions under different feeding levels, body size ranges, water temperature regimes, diet compositions, fish caloric values, etc.; these computer simulations will be useless without a quantitative description and a measure of the relative importance of these variables under field situations. Consequently, the purpose of our study was not to assess whether any single variable influenced fish growth, but to assess the relative importance of a suite of specific variables in explaining among-population variability in in situ fish growth rates. Our methodological approach was therefore to compare key variables among natural populations that varied significantly in terms of growth and not, as in laboratory experiments and bioenergetics model simulations, to manipulate given variables and quantify their influence on growth. We adopted the direct comparative approach because it is evident that most comparisons between treated and control fish (the experimental approach), in which a large range of variables are manipulated, will yield differences in growth rates between these fish. Similarly, changes to any of the numerous parameters of bioenergetics models will affect their output (most frequently predictions of growth or consumption rates). While the experimental and bioenergetics modelling approaches can be very useful as a source of hypotheses explaining among-population variability in fish growth rates, it is only through analyses of complete field data sets that these hypotheses can be thoroughly tested.

Body size — Hewett et al. (1991) argue that our use of relative growth and consumption rates was inappropriate to test the hypothesis of the existence of a significant positive relationship between these variables. They base their argument on the existence of an allometric relationship between consumption rate and body weight (Peters 1983). They note that such allometry can change the relative position of regression lines describing the relationship between growth and consumption rates for different sizes of fish. The displacement of regression lines seems to occur when the relationships are examined on a relative (grams per gram per day) rather than an absolute (grams per day) basis. Hewett et al. (1991) illustrate their point by sampling a total of three points from three growth/consumption relationships obtained using the Hewett and Johnson (1987) bioenergetics model (which is itself based on the existence of a strong positive relationship between fish growth and consumption rates) for age II+ fish of different initial sizes (10, 20, 39 g: Boisclair and Leggett 1989a). The analyses of these sample points is used to show that a very strong and positive relationship between fish growth and consumption rates, examined on an absolute basis (grams per day), may, in fact, appear weak and negative when evaluated on a relative basis (grams per gram per day). However, we argue that the sample points selected by Hewett et al. (1991) represent an extreme situation: (1) they are selected assuming that larger fish consume an increasing fraction of their maximum consumption rates, an assumption with little direct support for fish in the field, (2) they are taken from fish that have completed a different fraction of their growing season, and (3) they comprise fish that cover a size range larger than that observed in our study for age II+ fish. In our study, age II+ perch ranged from 5 to 15 g (compared with the 10–30 g used by Hewett et al. 1991) at the

TABLE 1. Relationships between growth (G) and consumption (C) rates (assessed on an absolute and relative basis) for fish ranging from 10 to 30 g (simulations performed by Hewett et al. 1991).

	Initial size (g)		
	10	20	30
Absolute basis ($g \cdot d^{-1}$)	$G = 0.25C - 0.075$	$G = 0.25C - 0.13$	$G = 0.26C - 0.19$
Relative basis ($g \cdot g^{-1} \cdot d^{-1}$)	$G = 0.039C - 0.0115$	$G = 0.38C - 0.0096$	$G = 0.36C - 0.0085$

beginning of the season and from 7 to 25 g (compared with the 15–40 g used by Hewett et al. 1991) at the end of the season. One exception to this pattern was age II+ perch from Lake Silver which grew from 19 to 49 g during the growing season.

It is clear that the conclusions reached using their figures 1 and 2 depend strongly on the points sampled. For instance, three different points selected by us from their simulations on an absolute basis (their fig. 1; third point of the 10-g fish, second point of the 20-g fish, first point of the 30-g fish) indicate the existence of a strong negative relationship between growth and consumption rates, while these same points, when analyzed on a relative basis (their fig. 2), suggest the existence of a strong positive relationship between these variables.

As we demonstrate below, even in the worst case situation presented by Hewett et al. (1991), the acceptance (or rejection) of the hypothesis of the existence of a significant positive relationship between growth and consumption rates is largely determined by the number of points sampled and not, as argued by Hewett et al. (1991), by the interference caused by the existence of an allometric relationship between consumption rate and fish weight.

To verify this point, we used the relationships produced by Hewett et al. (1991) to estimate the probability of supporting the hypothesis of the existence of a significant positive relationship between fish growth and consumption rates when such a relationship does exist. We estimated the relationships they obtained between growth and consumption rates on both an absolute and relative basis using their fig. 1 and 2 (Table 1). These relationships have two interesting characteristics: (1) the growth efficiency values calculated from their simulations on an absolute basis (25–26%) are different from those derived on a relative basis (36–39%), an artifact not encountered in our study, and (2) the growth efficiency values are approximately equal for fish which vary threefold in size. However, our own study (all age classes combined; Boisclair and Leggett 1989a) and that of many others (Paloheimo and Dickie 1968; Kerr 1971; Brett and Groves 1979; Condrey 1982) suggest that growth efficiency should decrease with fish size.

Despite these difficulties, a computer program was designed to select three sample points at random within the limits of the relationships between absolute and relative growth and consumption rates derived from Hewett et al. (1991). We tested the significance and calculated the coefficient (i.e. slope) of the relationship on both an absolute and relative basis. This procedure was repeated 1000 times. The sampling of three points from within the limits of the absolute relationships presented in Table 1 resulted in 821 insignificant relationships and 32 negative coefficients (Table 2a). Corresponding numbers on a relative basis were 733 and 15. All negative coefficients were associated with insignificant relationships. Consequently, as suggested by Hewett et al. (1991), the sampling of three points will lead to the rejection of the hypothesis of the existence of a significant positive relationship between growth and consumption rates 70–80% of the time, even when such a relationship does exist.

TABLE 2. Results of the 1000 simulations designed to estimate the probability of supporting the hypothesis of the existence of a significant positive relationship between fish growth and consumption rates as assessed on an absolute ($g \cdot d^{-1}$) and relative ($g \cdot g^{-1} \cdot d^{-1}$) basis when this relationship does, in fact, exist. (a) Simulations were performed using three points selected from within the range of relationships of growth and consumption rates derived using the Hewett and Johnson (1987) bioenergetics model (Table 1). (b) Simulations performed using 10 points selected from within the range of relationships of growth and consumption rates derived using the Hewett and Johnson (1987) bioenergetics model (Table 1).

	Minimum	Maximum	Average	Standard deviation
(a) 3 points sampled				
Absolute basis				
Coefficient (b)	−1.420	1.176	0.248	0.149
s_b	0.0001	2.050	0.092	0.135
Relative basis				
Coefficient (b)	−1.306	1.359	0.368	0.154
s_b	0.0002	2.214	0.095	0.139
(b) 10 points sampled				
Absolute basis				
Coefficient (b)	0.135	0.362	0.254	0.034
s_b	0.009	0.079	0.033	0.009
Relative basis				
Coefficient (b)	0.252	0.484	0.374	0.035
s_b	0.009	0.078	0.034	0.009

However, in our series of papers (Boisclair and Leggett 1989a, 1989b, 1989c, 1989d), the relationship between fish growth and consumption rates was tested using 10 points (i.e. populations). We therefore performed another series of 1000 simulations, each comprising 10 points selected within the limits of the relationships presented in Table 1. These relationships, either on an absolute or relative basis, were always significant at the $p < 0.05$ level (Table 2b). Furthermore, the coefficients of these relationships were always positive (Table 2b).

Our simulations, contrarily to the argument of Hewett et al. (1991), indicate that the conclusion reached following our analytical procedure was not influenced by the interference caused by the allometry between food consumption rate and fish weight. As such, our simulations clearly demonstrate that the probability of obtaining the correct conclusion regarding a preestablished significant positive relationship between fish growth and consumption rates is 1, either on an absolute or a relative basis, when, as in our field study, 10 points are selected within the limits of observed growth and consumption rates.

Finally, it is worth noting that had the influence of the allometry between consumption rates and fish weight produced the bias described by Hewett et al. (1991), one would expect the occurrence of even more relationships between growth and consumption rates when the data are analyzed by groups of narrower fish size classes. We performed such an analysis by groups of fish varying in mean weight by less than 5 g. No new relationships between fish growth and consumption rates were unveiled (Boisclair and Leggett 1990). These previous results, together with our analyses of worst case situations presented by Hewett et al. (1991), support the argument that our analytical procedure for testing the relationship between fish growth and consumption rates using relative rates was totally adequate.

Temperature — Hewett et al. (1991) approach the influence of water temperature from two different perspectives: (1) its potential influence on growth and (2) the potential relationship between consumption rates and water temperature. We deal with these questions sequentially.

We agree with Hewett et al. (1991) that a consistent water temperature difference of 3° has the potential (other variables being constant) to influence fish growth rates. However, the water temperature regime of the lakes we studied did not behave in a consistent pattern. Only two lakes were consistently warmer than the others; Lakes Bromont and Roxton were generally 2° warmer than the other lakes and were characterized by seasonal mean water temperatures (Bromont: 21.2°C; Roxton: 21.3°C) closer to optimum values for perch growth (22.5°C; Schneider 1973; Neill and Magnuson 1974). Furthermore, the fact that perch growth rates in Lake Roxton (age II+: 0.006; age III+: 0.004 g wet·g wet^{-1}·d^{-1}) were very close to the 12 lakes' average (age I+: 0.011; age II+: 0.005; age III+: 0.003 g wet·g wet^{-1}·d^{-1}), while those for Lake Bromont were below average (age I+: 0.008; age II+: 0.003; age III+: 0.002 g wet·g wet^{-1}·d^{-1}), despite the greater proximity of the average temperatures in these lakes to the optimal water temperatures for perch growth, suggests that the potential influence of water temperature on growth rates was masked by more important factors. Seasonal and average water temperatures in the remaining 10 lakes were always within ±1° of one another (Boisclair and Leggett 1989a). We therefore concluded that while it is possible that water temperature explained a fraction of the observed among-population growth differences, its importance was marginal compared with many other variables.

We concur with the comments of Hewett et al. (1991) regarding the potential relationship between consumption rates and water temperatures. However, the simulations performed by Hewett et al. (1991) support our argument that the influence of the among-lake water temperature differences explained little, if any, of the quantity of food consumed by fish in the lakes studied. As emphasized by their simulations, even a consistent water temperature difference of 3°, which exceeded the water temperature differences among our study lakes, results in a maximum of 24% change in food consumption rates. In the light of their own simulations, we question their interpretation that a ±1°, or even a consistent 3° water temperature difference, could explain an important fraction of the observed fourfold among-population variability in food consumption rates (Boisclair and Leggett 1989a). It is important to note also that the purpose of our study was not to explain among-population variability in consumption rates. From our perspective, the relevance of their point regarding the importance of the potential relationship between consumption rates and water temperature to the major objectives and conclusions of our study remains unclear.

Caloric density — It is generally recognized that energy (calories or joules) is the most appropriate currency with which to examine the performance of organisms and systems (van Valen 1976) and, as emphasized by Hewett et al. 1991), energy units should be used to balance bioenergetics models. The estimation of food consumption by the Hewett and Johnson (1987) computer model is based, in part (while all other variables are held constant), on the relationship between growth and prey caloric density. A given weight increment (growth) can be achieved by consuming either less of high-energy prey items or more of low-energy prey items. Consequently, bioenergetics models must account for both absolute variations in prey caloric densities and the timing between these variations and those of weight increments to accurately predict food consumption rates.

We agree with Hewett et al. (1991) that our interpretation that prey caloric density did not vary significantly among dates, populations, or age classes was not supported by our own analyses. We also concur with their comment that seasonal food consumption estimates derived from bioenergetics models using constant prey caloric density, while this value does in fact vary, might depart from true values. However, in our study, food consumption estimates derived using the Hewett and Johnson (1987) computer model were made while the model was implemented with date-, population-, and age-class-specific estimates of the caloric density and of the percent contribution of the 10 most important prey taxonomic groups (the maximum number of taxonomic groups allowed by the computer model). The percent contribution of the 10 most important prey taxonomic groups for any given sampling date, population, and age class was taken from Boisclair and Leggett (1989b), while their caloric density was derived from Cummins and Wuycheck (1971). Contrarily to the concerns expressed by Hewett et al. (1991), the food consumption estimates we derived using the Hewett and Johnson (1987) computer model accounted for among-date, -population, and -age-class variations in prey caloric density.

It is important to note that the series of assumptions and the procedure we adopted regarding the relative caloric densities of perch and their prey had a much smaller influence on the food consumption estimates we derived using the Hewett and Johnson (1987) computed model than that mentioned by Hewett et al. (1991). In our simulations, perch caloric density was set at 4677 cal·g dry^{-1} (Cummins and Wuycheck 1971). This value represents the low end of the range suggested by Craig (1977). The fact that most prey caloric density values ranged from 4000 to 4500 cal·g dry^{-1} (Cummins and Wuycheck 1971; Boisclair and Leggett 1989b and unpublished data) suggests that the average difference between perch and prey caloric density was in the order of 5–15%. These comparisons therefore indicate that, under our set of assumptions (while all other variables are held constant), food consumption estimates derived using bioenergetics models based on grams instead of calories would differ by approximately 5–15% and not by 55–100% as suggested by Hewett et al. (1991).

One purpose of our work was to quantify the degree of similarity between field-derived consumption rates that varied fourfold among populations and predictions of food consumption rates made by the Hewett and Johnson (1987) bioenergetics model for these populations. However, the precision of bioenergetics models is presently not sufficient to allow the sta-

tistical discrimination of two predicted food consumption rates that differ by less than 100% (Bartell et al. 1986). Furthermore, the statistical significance of the difference between two in situ food consumption values can be established when these estimates vary by at least 30% (Boisclair and Leggett 1988). Consequently, the statistical significance of a 5–15% difference between two consumption estimates derived using bioenergetics models is not only very difficult to establish, but can be regarded, for the purposes of our work, as trivial.

Comparison of consumption and growth rates — Hewett et al. (1991) apparently draw no distinction between analytical approaches (the suite of steps adopted to analyze a data set) and methodological approaches (the suite of steps adopted to collect the data). While we believe that the arguments presented above reject the reasoning of Hewett et al. (1991) that our analytical approach was flawed, we agree completely with their point that the [methodological] "approach taken by Hayward and Margraf (1987) is the best available method for comparing growth and consumption between populations of fish." The best expression of our agreement is that we used a similar direct comparative approach throughout our own work. Our study was based on in situ direct estimates of growth, food consumption rates, the caloric density of prey in the diets of the fish we studied, the percent contribution of different prey taxonomic groups and size classes to fish diets, and the numerical density and biomass of each fish species. We also determined the water temperature regime of each lake studied. Our methodological approach differed from that of Hayward and Margraf (1987) in only two ways: (1) they compared the growth rates of fish from only two habitats (eastern and western basins of Lake Erie) and (2) they estimated food consumption rates (a) using stomach evacuation rates predicted from water temperature or (b) using the Kitchell et al. (1977) bioenergetics model. These differences, however, have important implications. First, as demonstrated above (see Body size), the confidence in the conclusion of the presence (or absence) of a significant relationship between fish growth is low when $n = 2$. Second, and as pointed out in Boisclair and Leggett (1990) and by Hewett et al. (1991), the prediction of evacuation rates using only water temperature can lead to substantial errors in consumption rate estimates. Third, it is questionable whether one should seek to test the possibility of the existence of a significant positive relationship between fish growth and consumption rates when some of the required food consumption estimates are derived from the Kitchell et al. (1977) bioenergetics model which assumes the existence of such relationship.

Our work strongly suggests that the only acceptable methodological approach to the testing of hypotheses regarding the influence of biotic and/or abiotic factors on fish growth in situ is to directly estimate fish growth and the factors influencing it under numerous and differing field situations. It is comforting to note that this view is also shared by Hewett et al. (1991).

Models of Gastric Evacuation

It is generally recognized that fish evacuation rates are most directly influenced by water temperature (Molnar et al. 1967; Shraple et al. 1969; Brett and Higgs 1970; Tyler 1970; Edwards 1971; Elliott 1972; Steigenberger and Larkin 1974) and meal size (Noble 1973; Jobling 1981). Several other studies have also demonstrated the influence of food characteristics such as the size of food particles (Swenson and Smith 1973; Mills et al. 1984) and chemical composition of the food consumed (percentages of chitin, $CaCO_3$, and fat, food caloric value; Windell et al. 1969, 1972; Western 1971; Kionka and Windell 1972; Elliott 1972; Fange and Grove 1979) on evacuation rates. Finally, the influence of feeding schedules (Adron et al. 1973; Rosenthal and Paffenhofer 1972; Goddard 1974) and of fish body size (Pandian 1967; Grove et al. 1978; Mills et al. 1984) on evacuation rates have also been clearly established.

Interestingly, many experiments have obtained contradictory conclusions. For instance, while Hunt (1960) found that evacuation rate decreased with fish size, Swenson and Smith (1973), Jones (1974), and Jobling et al. (1977) concluded that evacuation rate increase with fish size, and Elliott (1972) and Seaburg and Moyle (1964) suggested that digestion rate was independent of body size. Furthermore, many experiments have defined evacuation rates using different units and models (hours, grams per hour, per hour; linear or exponential model) which, we believe, might have contributed to some confusion. Given the number of factors that seem to have a more direct influence on evacuation rate than body size and the contradictory results relative to the influence of fish size on evacuation rate, we believe that the correction suggested by Hewett et al. (1991) may be too simplistic. Taken together, studies designed to assess the effect of given variables on fish evacuation rates indicate that evacuation rate is influenced by too many factors to be accurately predicted for one another. At the very least they are unlikely to be predicted accurately enough to be useful to estimate food consumption rates under field situations.

We verified this point by executing a series of preliminary evacuation rate experiments (Boisclair and Leggett 1988). From these 18 evacuation rates, we assessed the influence of water temperature, fish weight, fish numerical density, and the percent contribution of plankton to fish diet on perch evacuation rate (Boisclair and Leggett, unpublished data). Only water temperature ($F_{1,14} = 36.46$; $p < 0.0001$; $R^2 = 0.64$) and the percent contribution of plankton to fish diet ($F_{1,14} = 7.15$; $p < 0.018$; $R^2 = 0.12$) explained a significant proportion of evacuation rate variability ($F_{2,14} = 22.78$; $p < 0.0001$; $R^2 = 0.76$). While the regression was highly significant, the 95% confidence interval around any prediction from that model was equivalent to $\pm 200\%$ of the mean. We therefore decided that an in situ digestion experiment should be performed on each sampling date (Boisclair and Leggett 1989a). This procedure allowed the influence of water temperature, meal size, and prey characteristics to be integrated into the estimate of digestion rate (and therefore into the estimate of ingestion rate) for that population and sampling interval.

Our conclusion, that the influence of fish size on perch evacuation rate is minimal, is supported by one of the few published experiments designed to test the influence of perch body size on evacuation rates within fish size ranges that approach those encountered during our field study. Persson (1981) estimated evacuation rates of 121- to 160-mm perch held at 21.7°C to range from 0.243 to 0.253. Corresponding estimates for perch from 89 to 104 mm ranged from 0.241 to $0.249 \cdot h^{-1}$. Similar experiments performed at 17.0°C showed that the evacuation rate of 120- to 162-mm perch was $0.31 \cdot h^{-1}$ compared with $0.12 \cdot h^{-1}$ for 92- to 110-mm perch in our study. Our analyses, together with those of others, suggest that while fish size may account for some variability in evacuation rate, its importance compared with water temperature, meal size, and prey type, which were accounted for in our study, is minimal.

There are presently two main routes available for those who wish to estimate the quantity of food consumed by fish: (1) the

direct route which requires the estimation of digestive tract (stomach plus intestine) evacuation rate and the quantity of food found in the digestive tract at the time of sampling (Eggers 1977; Elliott and Persson 1978) and (2) the indirect route of bioenergetics models which appear to require the measurement of everything but the estimation of stomach or digestive tract evacuation rate (Kitchell et al. 1977; Hewett and Johnson 1987). Hewett et al. (1991) seem to imply that the models designed to directly estimate the quantity of food consumed by fish (Eggers 1977; Elliott and Persson 1978) are based on the same physiological measurements as those used to construct bioenergetics models. We strongly disagree with this perspective. No term in existing bioenergetics models directly represents evacuation rates or the quantity of food found in either the stomach or the total digestive tract of fish. We recognize, however, that both approaches are based on physiological variables and that none provides the true consumption rate, a datum known only by those with gills! Furthermore, it is clear that the different perspectives regarding which route provides values closer to the "true" consumption rate are at the center of the current debate. From our perspective, the variables required by both direct and indirect approaches are subject to errors. We would argue, however, that given the choice between the estimation of evacuation rate and the weight of food found in the digestive tract (required by the direct approach) versus the requirement for estimates of growth rate, water temperature, the percent contribution of prey taxonomic groups to fish diet, prey caloric value, fish caloric value, the value of the undigestible fraction of the prey in the diet, plus approximately 20 model parameters (not to mention the untested assumption regarding fish activity required by the indirect approach), it might be prudent and more efficient to select the direct approach which requires fewer variables, parameters, and assumptions.

Activity Level

Hewett et al. (1991) incorrectly concluded that we estimated fish activity rates by substituting seasonal average values of fish growth, consumption rates, and water temperature into the Kitchell et al. (1977) bioenergetics model. In fact, both the Kitchell et al. (1977) and the Kerr (1982) bioenergetics models were iterated on a daily basis. We acknowledge, however, that the section in our paper in which we described our methodology was rather short: "We used a linear interpolation (Kitchell et al. 1977; Hewett and Johnson 1987) to define model values between the four sampling dates." (Boisclair and Leggett 1989d).

For illustrative purposes, assume that each lake was sampled at intervals of 25 d during a 75-d period (sampling on days 1, 25, 50, and 75 for a total of four sampling dates). Water temperature, fish weight (and growth), and consumption on days 2, 3, 4, 5,...,75 were estimated by linear interpolation between the sampling days, an approximation also utilized by Hewett and Johnson (1987). These values were used to derive all physiological compartments of the fish bioenergetic budget (growth, food consumption, standard metabolism, standard dynamic action, urinary and fecal losses) on a daily basis using the Kitchell et al. (1977) bioenergetics model. Our simulations and activity cost estimates (ingestion — all expenditures with the exception of activity; Boisclair and Leggett 1989d) therefore accounted for nonlinear effects and interactions of weight and temperature on metabolic processes with which Hewett et al. (1991) expressed unwarranted concern.

Conclusions

The comparison of our work with that of Hayward and Margraf (1987), together with the analyses performed in this paper and by Boisclair and Leggett (1990) and Hewett et al. (1991), suggests that the "better methods of making interpopulation (or age-class) comparisons of growth and consumption" referred to by Hewett et al. (1991) should (a) involve the analysis of more than two or three points (lakes or populations) and (b) be based on direct estimates of a suite of relevant variables including food consumption rates. The latter component should be added to the list of variables "to account for" presented by Hewett et al. (1991). Interestingly, this "better method" corresponds exactly to the methodological approach we selected in our series of papers (Boisclair and Leggett 1989a, 1989b, 1989c, 1989d).

We agree with Hewett et al. (1991) that "What if" types of questions are essential when exploring the potential for a given variable to influence fish growth rates. In fact, every hypothesis tested in our own work is derived from analyses and experiments which respond specifically to "What if" questions. However, we believe that it might also be useful to direct some research effort toward "How much" types of questions. From the very first paper of our series, the central questions addressed by our work were "How much do food consumption rates, diet composition, fish community characteristics, etc., vary among fish populations that significantly differ in growth rates " and "How much of the among-population variability of fish growth rates in the field is explained by food consumption, diet composition, fish community characteristics, etc.?" These question, examined in a multivariate framework (Boisclair and Leggett 1989a, 1989b, 1989c), resulted in the conclusion that food consumption explained on average 30% of among-population variability in fish growth rates compared with 60% for the total fish community numerical density (Boisclair and Leggett 1989c). Our analyses further suggested that among-population variability in fish growth rates might be determined by the balance between food consumption and activity expenditures (Boisclair and Leggett 1989d). This suggestion is not surprising per se, since bioenergetics equations represent growth as the result of food consumption minus all other costs including activity costs. However, our results contrast with some previous interpretations by indicating that activity costs may well represent an important and variable component of fish bioenergetics budgets (Boisclair and Leggett 1989d). It is worth noting, however, that Kerr (1971) and others (Wankowski and Thorpe 1979; Mittelbach 1983; Hart and Connellan 1984) have reached similar conclusions from a different perspective.

Bioenergetics models were developed (1) to serve as a conceptual framework (i.e. to ask "What if" types of questions) and (2) to allow managers to make predictions, often, about the quantity of food consumed by fish ("How much" types of questions). If, as suggested by our work (Boisclair and Leggett 1989d), activity costs do comprise a significant and variable proportion of the bioenergetic budgets of fishes, the use of a constant metabolic multiplier ((Standard metabolic rate + activity costs)/standard metabolic rate), now a common assumption in bioenergetics models applied to fishes, it is likely that the output of these models will depart regularly, and significantly, from true food consumption rates (Boisclair and Leggett 1989d). We agree with Hewett et al. (1991) that the effect of differences in consumption rates on growth rates observed in our study (Boisclair and Leggett 1989a, 1989b,

1989c, 1989d) remains unresolved. However, our work does suggest that one way to resolve this problem would be to evaluate the importance of fish activity rates on growth processes. The fact that no equation or submodel currently exists with which to account for the magnitude and variability of activity costs does not justify the *assumption* that this component is a constant multiplier of standard metabolic rate. This approach is contrary to the first raison d'être of bioenergetics models: to serve as a conceptual and creative tool. We recognize that "what if" types of questions can enhance one's understanding of growth processes." In this perspective, what if, as suggested by Boisclair and Leggett (1989d), fish activity costs do represent an important and variable component of bioenergetics budgets of fish. The likely possibility that fish activity does constitute an independent and significant component of fish bioenergetics models opens a new and exciting field of research. It is our contention that we should not try to hide this reality — D. Boisclair, *Département des sciences biologiques, Université de Montréal, C.P. 6128, Succursale «A», Montréal (Québec) H3C 3J7, Canada*, and W. C. Leggett, *Department of Biology, 1205 Ave. Dr. Penfield, Montreal, Que. H3A 1B1, Canada.*
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OBITUARY/NÉCROLOGIE

Lewis Rodman Day

1915–1990

Lew Day, internationally known fishery scientist and administrator, who served 17 years as Executive Secretary of the International Commission of the Northwest Atlantic Fisheries (ICNAF) and its successor, the Northwest Atlantic Fisheries Organization (NAFO), died suddenly on 7 May 1990 while undergoing heart surgery in Montreal's Royal Victoria Hospital.

Lew was born in Harrowsmith, Ontario, on 23 April 1915. He attended Queen's University, Kingston, and graduated in 1939 with an Honours BA in biology. In the fall of 1939, he enrolled in a graduate program in the University of Western Ontario at London. Under the supervision of Professor Helen Battle, he studied the embryology of the brook trout and was awarded his MA in 1941. His thesis was entitled "Organogenesis of the opercular apparatus in *Salvelinus fontinalis* (Mitchill)." Lew then registered in a Ph.D. program in zoology (fisheries) under the supervision of Professor J. R. Dymond at the University of Toronto in 1941. Already a member of the Canadian Officers Training Corps (COTC) contingent at the University of Toronto, he postponed graduate training and in 1942 joined the Canadian Army.

On 1 May 1943, he married Nursing Sister Ray Leslie (Monty) Montgomery in a military wedding ceremony at the Officers Training Establishment in Brockville, Ontario. A few months after the wedding in 1943, he was posted overseas, a lieutenant in the Canadian Army Service Corps. After service in England and Northwest Europe, he returned to Canada in 1945.

During his graduate work at the University of Western Ontario, Lew had worked as a summer student and student assistant for the Fisheries Research Board of Canada, then the research arm of the federal Department of Fisheries. Lew's graduate supervisor at the University of Western Ontario, Dr. Helen Battle, worked as a volunteer investigator and visiting scientist with the Fisheries Research Board of Canada at the Biological Station in St. Andrews, New Brunswick. Dr. Battle encouraged many of her students to seek summer employment with the Fisheries Research Board of Canada for training and experience. Thus, Lew worked out of the Biological Station at St. Andrews as a student assistant in 1940 and 1941, often in the field on salmon investigations on the Margaree and Moser rivers in Nova Scotia. It was natural, therefore, that on his return from overseas duty in 1945, he would turn again to fisheries work. That same year, 1945, he joined the scientific staff of the Biological Station as a zoologist and became a member of the Atlantic Herring Investigation Committee. The committee was chaired by Dr. Alex Leim and headquartered in St. Andrews. At that time the Atlantic herring was considered to be an underutilized species and the Atlantic Provinces and the Federal Government had joined forces to study ways of making better use of the herring stocks. Lew's co-workers on the herring investigation work, in addition to Dr. Leim, were scientists who were to become well known in their own special fields — W. B. Bailey, H. B. Hachey, L. Lauzier, S. Noel Tibbo, and R. W. Trites. In 1948, Lew went on the permanent staff of the Biological Station as a biologist and scientific assistant to the Director, Dr. A. W. H. Needler. Lew served in this capacity until 1955, when he was appointed Assistant Director under the new Director, Dr. John Hart. He served in this position until 1963.

During his years at the St. Andrews station, Lew worked on many fishery research programs in addition to the Atlantic herring investigation. He wrote some thirty scientific papers on such topics as lampreys, sharks, smelt, alewives, killifishes, and the bluefin tuna and swordfish fisheries, often in collaboration with colleagues.

In 1963, Lew and Monty moved from St. Andrews to Halifax, Nova Scotia. Lew had successfully competed for the

position of Executive Secretary to ICNAF, a position of considerable responsibility. ICNAF was formed in 1949 to make possible the best utilization of the fisheries resources of the area through the cooperation of the ten participating nations. Lew served as Executive Secretary of ICNAF for 17 years from 1963 to 1979 and as the first Executive Secretary of its successor, NAFO, from January 1980 until his retirement in July the same year.

Lew Day's service to ICNAF was exemplary and highly regarded by all who worked with him. A recent comment by a former colleague and fellow scientist, Dr. Art May, perhaps sums up best the high regard accorded this quiet Canadian who took the executive role of a major international commission. Art May wrote... "He fulfilled this role with dedication, with style, with flair, with tact, with charm, and above all with quiet competence...He was the model of an international public servant" (1990. J. Northwest Atl. Fish. Sci. 10).

After retirement in July 1980, Lew and Monty returned again to St. Andrews, bought a house on Charles Street, and entered into the many activities that characterize a small town. Because of his interest in scientific literature, he was approached in 1981 to take on the index editor's responsibilities for preparation of the annual index of publications of the Department of Fisheries and Oceans, contracted to the Huntsman Marine Science Centre. Lew accepted and continued this work despite ill health until April 1990.

An avid golfer, Lew was an active member of the Algonquin Golf Club, was past president, Chairman of Junior Development, and was a member of the Board of Directors. He was an active choir and Session member of Wesley United Church and a member of the Royal Canadian Legion. He was also a past president of Sunbury Shores Arts and Nature Centre and served as chairman of the St. Andrews Bicentennial Committee.

Recurring illness made the last few years difficult for Lew and Monty, but Lew carried on with his own special calm affability and optimism. He is survived by his wife Monty, daughters, Maureen and Nancy, and brothers Albert and Kenneth.

Lew joins a long and honourable list of Canadian fisheries scientists who have contributed with great distinction to our knowledge and administration of one of the most significant natural resource industries in the world. His contributions will long be remembered.

W. B. Scott
Huntsman Marine Science Centre
St. Andrews, N.B.

ANNOUNCEMENTS/AVIS

Symposium Announcement

Aquatic Ecology: Scale, Pattern & Process

The first joint meeting of the British Ecological Society (BES) and the American Society for Limnology and Oceanography (ASLO), 5–8 April 1992, at University College, Cork, Ireland.

- An international programme of speakers plus offered poster sessions
- An emphasis on integration across scales and between marine and freshwater habitats

For further details and to place your name on the mailing list, write to Dr. P. S. Giller, Department of Zoology, University College, Lee Maltings, Prospect Row, Cork, Ireland.

Annonce d'un symposium

Aquatic ecology: scale, pattern & process

Première assemblée conjointe de la British Ecological Society (BES) et de l'American Society for Limnology and Oceanography (ASLO) du 5 au 8 avril 1992, University College, Cork, Irlande.

- Des conférenciers viendront de tous les pays, et certains participeront à des séances d'affichage
- On insistera sur les rapports entre les systèmes de mesure, et entre les habitats d'eau douce et les habitats marins

Vous êtes priés d'écrire à l'adresse suivante pour obtenir des renseignements complémentaires et faire inscrire votre nom sur la liste d'envoi : Dr. P. S. Giller, Department of Zoology, University College, Lee Maltings, Prospect Row, Cork, Irlande.

Canadian Conference for Fisheries Research (CCFFR)

Dalhousie University
Halifax, Nova Scotia
January 3–4, 1992

Announcement of Conference and Call for Papers

This conference will be held in Halifax, N.S., January 3 and 4, 1992. The major themes of the conference will be (1) Large-Scale Environmental Effects on Fish Survival and Abundance, (2) Role of the Early Life History Stage in Population Dynamics, and (3) Migration, Mixing and Dispersal in Fish and Invertebrate Stocks.

While papers on other themes may be submitted, authors are encouraged to adhere to the above themes as much as possible.

Deadline for abstracts is August 30, 1991. They should be typed (maximum 200 words, single spaced) and nature of presentation (oral/poster) indicated. A high quality of copy is required, suitable for photographic reproduction.

Abstracts should be addressed to Dr. Steve Campana, Bedford Institute of Oceanography, Biological Sciences Branch, Department of Fisheries and Oceans, P.O. Box 1006, Dartmouth, Nova Scotia B2Y 4A2, Canada. Telephone: (902) 426-3233.

Conférence canadienne sur la recherche sur les pêches (CCRP)

Université Dalhousie
Halifax, Nouvelle-Écosse
les 3 et 4 janvier 1992

Avis de conférence et demande d'articles

Cette conférence aura lieu les 3 et 4 janvier 1992 à Halifax (N.-É.). La conférence portera sur les principaux thèmes suivants : 1) Conséquences environnementales à grande échelle sur la survie et l'abondance des poissons, 2) Rôle du stade primaire du cycle vital dans la dynamique des populations et 3) Migration, mélange et dispersion des stocks de poissons et d'invertébrés.

On peut présenter des études sur d'autres thèmes quoiqu'on encourage les chercheurs à s'en tenir autant que possible aux thèmes susmentionnés.

La date limite d'envoi de résumés a été fixée au 30 août 1991. La résumé doit être dactylographié (200 mots au maximum, simple interligne) et l'on doit préciser le genre de présentation (présentation orale/affiche). Une copie de très bonne qualité est requise pour faciliter la reproduction photographique.

Veuillez faire parvenir votre résumé à Dr. Steve Campana, Institut Océanographique de Bedford, Direction des Sciences biologiques, Ministère des Pêches et des Océans, C.P. 1006, Dartmouth (Nouvelle-Écosse) B2Y 4A2, Canada. Téléphone : (902) 426-3233.

Announcement and Call for Papers

International Symposium on Fish Otolith Research and Application

24–27 January 1993
Hilton Head, South Carolina, USA

Otoliths have long been recognized for their utility in determining the age of fish. Over the past decade, studies of daily growth rings, otolith composition, and otolith shape have revolutionized the study of population dynamics, stock structure, migration, and environmental dating in both young and old fish. In addition, it is now possible to economically and permanently mass-mark the otoliths of millions of young hatchery-reared fish before release to the wild. The objectives of this symposium are to bring together those who study otoliths and those who use them as tools, in order to assess knowledge, clarify issues, and develop options and opportunities in this rapidly evolving field. Emphasis will be placed on innovative approaches to otolith research and their application in fish biology and population dynamics studies.

Over the four days of the symposium, invited lectures will be used to develop several theme sessions:

Otolith Growth, Morphology, and the Estimation of Fish Growth, focusing on studies of physiology and function, growth backcalculation, periodic features (daily, annual, and other), age validation, and utility for stock identification.

Otoliths in Studies of Population Biology, emphasizing otolith use in recruitment and population dynamics studies, hatchdate distributions, mass-marking techniques, use as indices of dispersal and migration, and statistical approaches to growth and mortality estimation.

Otolith Composition as an Index of Environmental History, focusing on microchemistry, use of isotopes to determine chronologies, pollution monitoring, and stock identification.

In addition, there will be workshops on otolith techniques, as well as hands-on clinics dealing with image analysis sytems, in vitro otolith studies, and detection of marked otoliths.

Selected papers from both the invited and contributed presentations will be published in a peer-reviewed book.

The Steering Committee is now soliciting contributed presentations for the symposium. Titles and abstracts (to a maximum of 200 words) should be submitted **before 30 April 1992.** To be placed on a mailing list for further information, please contact one of the following convenors: Dr. S. E. Campana, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada; telephone (920) 426-3233, FAX (902) 426-2256; Dr. J. M. Dean, Belle W. Baruch Institute, University of South Carolina, Columbia, SC 29208, USA; telephone (830) 777-3932, FAX (803) 777-3935.

Annonce et demandes de documents

Symposium international sur la recherche sur les otolithes de poissons et ses applications

24–27 janvier 1993
Hilton Head, Caroline du Sud (États-Unis)

On reconnaît depuis longtemps l'utilité des otolithes pour la détermination de l'âge des poissons. Au cours de la dernière décennie, les études portant sur les anneaux de croissance journalière, sur la compositon des otolithes et sur l'analyse des contours ont révolutionné l'étude de la dynamique des populations, de la structure des stocks, de la migration et l'analyse de l'histoire environnementale, autant dans le cas des jeunes que des vieux poissons. De plus, il est maintenant possible de marquer en masse, de façon économique et durable, les otolithes de millions de jeunes poissons élevés en bassin avant de les relâcher en milieu naturel. L'objectif de ce symposium est de réunir à la fois ceux qui étudient les otolithes et ceux qui les utilisent comme outils de travail, dans le but d'évaluer l'état des connaissances, de clarifier certaines questions et de développer des options et perspectives dans ce domaine qui évolue rapidement. Une importance particulière sera accordée aux approches innovatrices dans le domaine de la recherche sur les otolithes ainsi qu'à leurs applications en biologie des poissons et en dynamique des populations.

Au cours des quatre jours que durera le symposium, des exposés de conférenciers invités serviront à introduire les thèmes de diverses scéances :

Croissance de l'otolithe, morphologie et estimation de la croissance du poisson : ce thème sera axé sur les études physiologiques et fonctionnelles, le rétro-calcul de la croissance, les caractéristiques périodiques (journalières, annuelles et autres), la validation de l'âge et l'utilisation à des fins d'identification de stocks.

Les otolithes dans les études de biologie des populations : on mettra l'emphase sur le recrutement et les études de dynamique des populations, la distribution des dates d'éclosion, les techniques de marquage en masse, l'utilisation comme indices de dispersion et de migration, ainsi que sur les approches statistiques d'estimation de la croissance et de la mortalité.

Composition de l'otolithe en tant qu'indicateur de l'histoire environnementale : l'accent sera mis sur la microchimie, l'utilisation des isotopes dans la détermination de la chronologie des températures, le monitoring de polluants et l'identification de stocks.

De plus, il y aura des ateliers de travail sur les techniques liées aux otolithes, ainsi que des scéances pratiques portant sur les systèmes d'analyse d'images, l'étude d'otolithes in vitro et la détection des traces de marquage sur les otolithes.

Des documents sélectionnés parmis ceux qui auront été soumis seront réunis dans une même publication qui aura au préalable été examinée par un comité de révision.

Le comité organisateur est présentement en période de sollicitation d'exposés pour le symposium. Les titres et résumés (d'au plus 200 mots) devront être soumis avant le **30 avril 1992.** Les personnes désirant être inscrites à la liste d'adresses afin de recevoir de plus amples informations sont priées de contacter l'un des présidents du comité : Dr. S. E. Campana, Institut océanographique de Bedford, C.P. 1006, Dartmouth (N.-É.) B2Y 4A2, Canada; téléphone (902) 426-3233, FAX (902) 426-2256; Dr. J. M. Dean, Belle W. Baruch Institute, University of South Carolina, Columbia, SC 29208 (É.-U.); téléphone (803) 777-3932, FAX (803) 777-3935.

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